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(71) Applicant (*for all designated States except US*): **BIOGNOSTIK GESELLSCHAFT FÜR BIOMOLEKULARE DIAGNOSTIK MBH** [DE/DE]; Gerhard-Gerdes-Str. 19, 37079 Göttingen (DE).

(72) Inventors; and

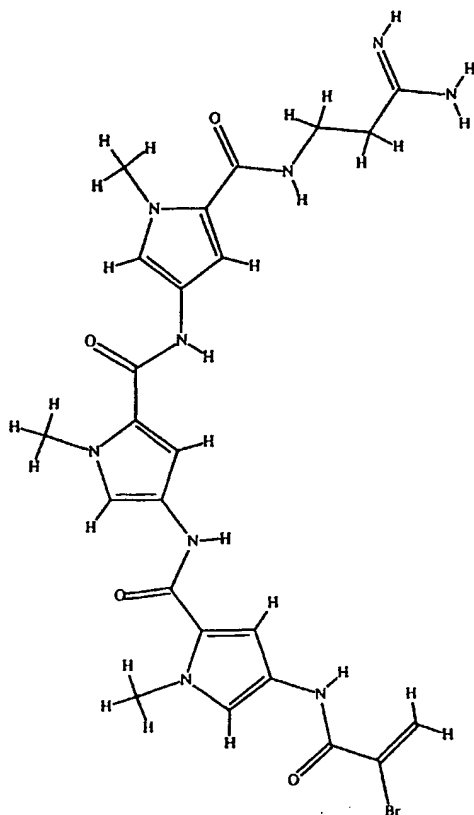
(75) Inventors/Applicants (*for US only*): **SCHLINGENSIEPEN, Karl-Hermann** [DE/DE]; Pappelweg 3, 37120 Bovenden-Lengeln (DE). **SCHLINGENSIEPEN, Reimar** [DE/DE]; Zur Scharfmühle 34, 37083 Göttingen (DE). **APFEL, Rainer** [DE/DE]; Eilsbrunner Str. 22, 93161 Sinzig (DE). **BRYSCH, Wolfgang** [DE/DE]; Brüder-Grimm-Allee 62, 37075 Göttingen (DE). **JACHIMCZAK, Piotr** [DE/DE]; Sterenstr. 37, 97074 Würzburg (DE). **BOGDAHN, Ulrich** [DE/DE]; Talblick 27, 93055 Regensburg (DE).

(74) Agents: **MEYERS, Hans-Wilhelm** et al.; Postfach 10 22 41, 50462 Köln (DE).

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[Continued on next page]

(54) Title: **A METHOD FOR REVERSING THE IMMUNOSUPPRESSIVE EFFECTS OF THE MELANOMA INHIBITORY ACTIVITY "MIA"**



(57) Abstract: A method for stimulating immune cells and/or the immune system, and/or reducing invasion and/or metastasis of tumor cells by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" MIA.

WO 01/68122 A2



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A method for reversing the immunosuppressive effects of theMelanoma Inhibitory Activity "MIA"

The polypeptide "Melanoma Inhibitory Activity", MIA, was discovered in 1989 as a factor that inhibits growth of melanoma tumor cells. The antiproliferative action of MIA was also demonstrated in other tumor cells and Peripheral Blood Mononuclear Cells. Thus, CANCER RES. 49; 5358-63, Bogdahn et al, (1989) demonstrated a very strong tumor cell growth-inhibiting effect of a factor called melanoma inhibitory activity (MIA). Three active fraction pools, named MIA-I, MIA-II and MIA-III were identified. Tumor stem cell colony formation was reduced by an astonishing 99.89% e.g. by MIA-II.

CANCER RES 50; 6981-86, Weilbach et al. (1990) further demonstrated that MIA inhibits cell proliferation by prolonging of the S-Phase and arrest of the cells in the G2 compartment.

PROC.AACR 40; 79, Jachimczak et al. (1999) further extended these observations to Peripheral Blood Mononuclear Cells (PBMCs), although to only a slight degree: "IL-2-stimulated PBMC proliferation has been only slightly inhibited by addition of MIA".

CANCER RES. 54; 5695-5701, Blesch et al. (1994) identified MIA as a 131-amino acid precursor, processed into a mature 107-amino acid protein. This publication confirmed that MIA acts as a potent tumor cell growth inhibitor for malignant melanoma cell and further extended this observation to other neuroectodermal tumors and concluded that, "... MIA ... might be attractive as a future antitumor therapeutical substance."

Controversial data were obtained regarding correlation of MIA expression with melanoma progression. CANCER RES. 57; 3149-53, Bosserhoff et al. (1997) and ANTICANCER RES. 19; 2691-3, Bosserhoff et al. (1997) found enhanced MIA levels in 13- 23% of stage I and II melanomas, but in 100% of stage III or stage IV disease.

In contrast, CANCER RES. 55; 6237-43, van Groningen et al. (1995) found MIA mRNA expression in non metastasising cell lines and an inverse

- 2 -

correlation of MIA mRNA expression with pigmentation in melanoma metastasis lesions, but notably expression was found to be absent in highly metastasising cell lines. Furthermore, CLIN. CANCER RES. 5; 1099-105 , Muhlbauer et al. (1997) concluded that „. . .MIA amplification seems to be of little value as a surrogate marker for clinical staging or the detection of metastatic disease."

Surprisingly while the above literature suggested that MIA had the potential as a therapeutic agent to treat melanoma patients it was now found that in contrast MIA is a potent immunosuppressive factor and that agents inhibiting expression and/or function of MIA have therapeutic potential for treatment of neoplasms and immunosuppression.

The present invention therefore pertains to a method for stimulating immune cells and/or the immune system, and/or reducing invasion and/or metastasis of tumor cells by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" MIA.

According to the invention the stimulation of the immune system is preferably achieved by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" MIA in combination with enhancing expression in target cells and/or target pathogens of the molecules listed under a) to m);

alternatively by vaccination with DNA and/or RNA coding for all or part of the molecules listed under a) to m) and/or polypeptides contained in the molecules listed under a) to m);

by transfection of an organism and/or transfecting the target cells and/or target pathogens with genes coding for the molecules listed under a) to m);

by applying to an organism and/or to the target cells and/or target pathogens the molecules listed under a) to m);

and/ or by enhancing the synthesis and/or function of molecules stimulating and/or enhancing and/or upregulating and/or positively regulating the immune response with molecules including the molecules listed under a) to m), wherein

- 3 -

- a) represents molecules selected from the group comprising chemokines, including lymphotactin and/or immune cell attracting factors;
- b) represents elements selected from the group comprising viruses and/or parts of viruses, including adeno viruses, papilloma viruses, Epstein-barr-Viruses, viruses that are non-pathogenic including Newcastle-Disease virus, Cow-pox-virus;
- c) represents molecules selected from the group comprising autologous and/or heterologous MHC-molecules;
- d) represents molecules selected from the group comprising molecules involved in antigen processing;
- e) represents molecules selected from the group comprising molecules involved in antigen presentation;
- f) represents molecules selected from the group comprising molecules involved in mediating immune-cell effects;
- g) represents molecules selected from the group comprising molecules involved in mediating immune cell cytotoxic effects;
- h) represents molecules selected from the group comprising molecules involved in antigen transportation;
- i) represents molecules selected from the group comprising co-stimulatory molecules;
- j) represents molecules selected from the group comprising peptides enhancing recognition by immune cell and/or cytotoxic effects of immune cells;
- k) represents molecules selected from the group comprising peptides containing one or more amino acids differing between a protein in the target cell from the other cells within an organism including, but not limited to antigens, specific for melanoma cells and/or melanocytes and/or breast cells and/or breast cancer cells;

- 4 -

according to the invention the inhibition of the syntheses and/or function of MIA is achieved by using molecule of group I) wherein

l) represents molecules selected from the group comprising the peptides according to j) being

peptides containing one or more mutations and/or amino acid substitutions of the ras protein amino, the p53 protein, the EGF receptor protein, fusion peptides and/or fusion proteins, the retinoblastoma protein, peptides containing one or more mutations and/or amino acid substitutions and/or amino acid substitutions caused by gene rearrangements and/or gene translocations, peptides containing one or more mutations and/or amino acid substitutions of proteins coded by oncogenes and/or protooncogenes, proteins coded by anti-oncogenes and/or tumor suppressor genes;

peptides derived from proteins differing in the target cell by one or amino acids from the proteins expressed by other cells in the same organism,

peptides derived from viral antigens and/or coded by viral nucleic acids,

peptides derived from proteins over expressed in the target cell compared to a normal cell

and combinations thereof

m) tumor cell extracts and/or tumor cell lysates and/or adjuvants.

In a preferred embodiment of the invention the inhibition of the expression and/or functional activity of MIA is achieved by using at least one nucleic acid molecule, peptide, protein or low molecular weight substance. Preferably, the nucleic acid molecule is an oligo- or polynucleotide molecule, in particular an antisense molecule and/or ribozyme.

Methods for preparing effective antisense oligonucleotides are known to those skilled in the art. A preferred method is disclosed in WO 99/63975, incorporated by reference.

The inhibition of the synthesis and/or function of MIA is preferably achieved by using at molecules comprising the following antisense sequences:

- 5 -

MIA-2841-W	GTC AGG AAT CGG CAG	(Seq. ID No 1)
MIA-1278-W	CTT GGA GAA GAC ATA C	(Seq. ID No 2)
MIA-2842-W	TGC CTC CCC AGA AG	(Seq. ID No 3)

as well as the following sequences (Seq. ID No 10-39):

AGCCATGGAGATAG  
CAGCCATGGAGATAG  
ACAGCCATGGAGATAG  
CACAGCCATGGAGATAG  
CCACAGCCATGGAGAT  
GCCATGGAGATAGG  
AGCCATGGAGATAGG  
CAGCCATGGAGATAGG  
ACAGCCATGGAGATAGG  
CATGGAGATAGGGT  
CATGGAGATAGGGTG  
CATGGAGATAGGGTGG  
ATGGAGATAGGGTG  
ATGGAGATAGGGTGG  
ATGGAGATAGGGTGGC  
ATGGAGATAGGGTGGCT  
GGAGATAGGGTGGC  
GGAGATAGGGTGGCT  
GAAATAGCCCAGGC  
GAAATAGCCCAGGCG  
GAAATAGCCCAGGCGAG  
GGAAATAGCCCAGG  
GGAAATAGCCCAGGC  
GTCTTCACATCGAC  
GTCTTCACATCGACT  
GTCTTCACATCGACTT  
GTCTTCACATCGACTTT  
GTCTTCACATCGACTTTG  
GTCTTCACATCGACTTTG  
CCATTTGTCTGTCTTCAC

or parts of the sequences having at least 8 nucleotides.

Methods for synthesizing further antisense oligonucleotides are for example disclosed in WO 98/33904 of the same applicant.

Preferably, the antisense and/or ribozyme molecule is derived by synthesising a sequence wholly or partially complementary to MIA mRNA and testing for inhibitory activity of MIA.

According to the invention the antisense and/or ribozyme molecule is for example integrated into a DNA delivery system, comprising viral and/or non-viral vectors together with lipids selected from the group of anionic lipids, cationic lipids, non-cationic lipids and mixtures thereof.

In a preferred embodiment of the invention the nucleic acid molecules contain flanking sequences and/or vector sequences and/or sequences enhancing the expression and/or transfection of the nucleic acid molecules. In a further preferred embodiment of the invention the nucleic acid molecules are part of one or more vectors and/or viral sequences and/or viral vectors.

According to the invention it is preferred that the antisense and/or ribozyme molecule is modified at one or more of the sugar moieties, the bases and/or the internucleotide linkages as well as the phosphate moieties. For example, the modification of the oligonucleotides, ribozymes and/or nucleic acids comprises modifications such as phosphorothioate (S-ODN) internucleotide linkages, methylphosphonate internucleotide linkages, phosphoramidate linkages, peptide linkages, 2'-O-alkyl modifications of the sugar, in particular methyl, ethyl, propyl, butyl and the like, 2'-methoxyethoxy modifications of the sugar and/or modifications of the bases. The various modifications may be combined in an oligo- or polynucleotide.

In a further preferred embodiment of the invention the oligonucleotides, ribozymes and/or nucleic acids are coupled to or mixed with folic acid, hormones, steroid hormones such as oestrogene, progesterone, corticosteroids, mineral corticoids, peptides, proteoglycans, glycolipids, phospholipids and derivatives thereof.

Inhibition of the expression and/or functional activity of MIA can also be achieved using peptides or proteins.

The peptide and/or protein that can be used in the method of the invention can be obtained by screening an expression library and testing the expression products for inhibiting expression and/or functional activity of MIA.



- 7 -

Alternatively, a synthetic peptide and/or protein can be obtained by screening randomly synthesised peptides and/or polypeptides for inhibiting expression and/or functional activity of MIA.

Suitable peptides binding to MIA are for example the following peptides (SEQ ID No. 40-63):

VPHIPPN  
MPPTQVS  
QMHPWPP  
QPPFWQF  
TPPQGLA  
IPPYNTL  
AVRPAPL  
GAKPHPQ  
QQLSPLP  
GPPPPSPV  
LPLTPLP

QLNVNHQARADQ  
TSASTRPELHYP  
TFLPHQMHPWPP  
VPHIPPNSMALT  
RLTLLVLIMPAP

RKLPPRPRR  
VLASQIATTPSP  
TPLTKLPSVNHP  
PPNSFSSAGGQRT  
EQDSRQGQELTKKGL

ETTIVITWTPAPR  
TSLNISWDAPAVT  
NSLLVSWQPPRAR

and proteins or peptides comprising the foregoing peptides and analogs or derivatives of these peptides.

EMBO J. 20; 340-349, Stoll et al. (2001) disclose the three-dimensional structure of human MIA, thus allowing the computational construction and synthesis of specific peptides binding to MIA.

In a further embodiment of the invention, the inhibition of expression and/or functional activity of MIA as well as of the expression of the MIA gene and/or MIA mRNA is achieved by using an inhibitor of low molecular weight which can

for example be obtained by combinatorial chemistry and testing the products for inhibiting expression and/or functional activity of MIA [Fernandes, P. B. in Curr. Opin. Chem. Biol. 1998; 2 (5): 597-803 Technological advances in high-throughput screening].

Low molecular weight molecules (small molecules) as used herein are molecules having up to 100 carbon atoms in combination with further atoms such as N, S, O, P and the like.

Suitable small molecules can also be identified using computational methods. Methods for computational construction are for example disclosed in Murcko, M. A. , Caron, P.R., Charifson, P.S. (1999), Structure-based drug design, Annual Reports in Medicinal Chemistry, vol. 34, Academic Press, San Diego, 1999.

Suitable compounds are for example structures 1 to 492 identified in Figures 1 to 42. Also structures, which comprise structures 1 to 492 as substructures are useful in the present invention. Also parts and/or substructures of the structures 1 to 492 are useful in the present invention, as long as they comprise at least an aromatic system and an amid-bond.

In a further embodiment of the invention, the inhibition of expression and/or functional activity MIA is achieved by using DNA or RNA derivatives including aptamers and/or spiegelmers that bind to MIA.

Inhibition of expression and/or functional activity of MIA is can also be achieved by using antibodies or antibody fragments, such as F<sub>ab</sub>-fragments, single chain antibodies or combinations thereof. These molecules can be identified and obtained by screening antibody libraries and testing the expression products for inhibiting expression and/or functional activity of MIA.

Any of the foregoing elements, molecules or substances can be combined with an immunostimulatory agent, for example cytokines and/or inhibitors of the expression and/or function of interleukin-10 and/or transforming growth factor beta (TGF- $\beta$ ) and/or Prostaglandin B2 and/or receptors for Prostaglandin E2 and/or inhibitors of VEGF.

The present invention is also concerned with a composition for the manufacturing of a medicament comprising a molecule or a combination of molecules which is able to inhibit the expression and/or functional activity of MIA.

The resulting medicament comprising an inhibitor of the expression and/or functional activity of MIA is also subject of the present invention. The medicament of the invention may be combined with an immunostimulatory agent.

Any of the foregoing elements, molecules or substances can be employed for the preparation of a medicament for the prevention or the treatment of neoplasms, infections and/or immunosuppressive disorders.

Preferably, both, the inhibitor of MIA expression and/or functional activity is applied locally to a tumor or other pathologically affected site or organ and may also is applied systemically (e.g. i.v. or s.c. or orally).

The present invention is also related with the use of a method for stimulating the immune system by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" (MIA) in combination with the use of methods and/or molecules enhancing the immune response against diseased cells or pathogens, methods and/or molecules enhancing immunogenicity of target cells and/or target pathogens and/or

immunostimulatory molecules, comprising cytokines including interleukins, including IL-1, IL-2, IL-4, IL- 12, IL-18, such cytokines being applied systemically to an organism including man or being applied locally e.g. to certain regions or organs or parts of organ or compartments of a body

and/or

enhancing expression of cytokines in target cells or pathogens by stimulating their expression and/or by transfecting expression Systems into the target cell or target pathogen, capable of expressing these cytokines

and/or

- 10 -

chemokines attracting immune cells including lymphotactin, such chemokines being applied systemically to an organism including man or being applied locally e.g. to certain regions or organs or parts of organ or compartments of a body

and/or

enhancing expression of chemokines in target cells or pathogens by stimulating their expression and/or by transfecting expression systems into the target cell or target pathogen, capable of expressing these chemokines

and/or

peptides and/or DNA and/or RNA molecules and/or other antigens that are found in tumor cells and/or pathogens, but not in normal cells and/or

enhancing expression of peptides and/or antigens that are found in tumor cells and/or pathogens, but not in normal cells

and/or

tumor cell extracts and/or tumor cell lysates and/or adjuvants.

The method of the present invention is especially useful for the treatment of

1. Solid tumors, e.g. cancer of the skin (including melanoma), head and neck cancer, sarcoma (including osteosarcoma and chondrosarcoma), retinoblastoma, breast cancer, ovarian cancer, small-cell bronchogenic/lung carcinoma, non-small-cell bronchogenic/lung carcinoma, esophageal cancer, colon carcinoma, colorectal carcinoma, gastric cancer, small intestine carcinoma, liver carcinoma, carcinoma of the kidney, pancreas carcinoma, gallbladder cancer, cervical carcinoma, endometrial cancer, mesothelioma, prostate carcinoma, testicular carcinoma, brain tumor

2. Leukemia, e.g. myeloid leukemia (acute and chronic), acute lymphoblastic leukemia (ALL), Non-Hodgkin Lymphoma, Hodgkin-Lymphoma

- 11 -

3. Degenerative disorders, e.g. arthritis, degeneration/injury of cartilage and bone
4. Immunosuppressive diseases e.g. HIV infection, myelosuppressive diseases, ataxia-telangiectasia, DiGeorge syndrome, Bruton disease, congenital agammaglobulinemia, combined immunodeficiency disease, Wiscott-Aldrich syndrome, complement deficiencies, leukopenia.

## Examples

### Example 1

Allogenic anti-glioma LAK immune response was strongly inhibited by exogenous addition of MIA.

To study the effects of MIA upon cytotoxic T-lymphocytes (CTL) and Lymphokine Activated Killer cells (LAK cells) a CARE-LASS assay has been employed (Lichtenfels, R., Biddison, W.E., Schulz, H., Vogt, A.B. and R. Martin. CARE-LASS (calcein-release assay), an improved fluorescence based test system to measure cytotoxic lymphocyte activity J. Immunol. Meth., 172: 227-239, 1994). Briefly, glioma cells were harvested, washed in 5%FCS/PBS solution and resuspended at 10 Mio cells/ml in 5%FCS/FBS. Calcein-AM was added to a final concentration of 25  $\mu$ M (Molecular Probes, USA). The cells were labeled for 30 min at 37 °C then washed twice in 5%FCS/PBS, adjusted to 1 Mio cells / ml and loaded into 96-well U-shaped microtiter plates at the final concentration of 0.1 Mio / 100  $\mu$ L / 1 well (Nunc, Denmark). To measure cytotoxic activity of effector cells pretreated with MIA (f.c. 500 ng/ml), wells were loaded with 100  $\mu$ L of CTL and LAK cells to produce the desired E:T ratios of 1:10 and 1:100. To measure spontaneous release and total release of calcein, wells were preloaded with 100  $\mu$ L 5% FCS/PBS or 100  $\mu$ L lysis buffer (50nM sodium-borate, 0.1% Triton® X 100, pH 9.0) respectively. After incubating the plate for 4 h at 37° C the supernatants (50  $\mu$ L) were transferred into new wells and measured using an automated fluorescence scanner

- 12 -

(Titertek Fluoroskan II, Germany). The percent cytotoxicity was determined from the following equation:

$$\frac{F/\text{CTL assay} - F \text{ spontaneous release}}{F \text{ total lysis} - F \text{ spontaneous release}} \times 100 = \% \text{ cytotoxicity}$$

#### Results:

MIA inhibited autologous and allogenic LAK cytotoxicity against malignant glioma cell lines (HTZ-17, -243, -374, -375) up to 40% compared to controls.

#### Example 2

Furthermore, inhibition of MIA synthesis in MIA-secreting melanoma cells enhanced autologous LAK and CTL activity.

To study the effects of MIA upon cytotoxic T-lymphocytes (CTL) and Lymphokine Activated Killer cells (LAK cells) a CARE-LASS assay has been employed as described above in Example 1.

Briefly, melanoma cells were harvested, washed in 5% FCS/PBS solution and resuspended at 10 Mio cells/ml in 5% PCS/PBS. Calcein-AM was added to a final concentration of 25  $\mu\text{M}$  (Molecular Probes, USA). The cells were labeled for 30 min at 37 °C, then washed twice in 5% FCS/PBS, adjusted to 1 Mio cells / ml and loaded into 96-well U-shaped microtiter plates at the final concentration of 0.1 Mio / 100  $\mu\text{l}$ /1 well (Nunc, Denmark). To measure cytotoxic activity of effector cells pretreated with MIA-antisense oligonucleotides (f.c. 1-5  $\mu\text{M}$ ), wells were loaded with 100  $\mu\text{l}$  of CTL and LAK cells at E T ratios of 1:10 and 1:100. To measure spontaneous release and total release of calcein, wells were preloaded with 100  $\mu\text{l}$  5% FCS/PBS or 100  $\mu\text{l}$  lysis buffer (50 nM sodium-borate, 0.1% Triton® X 100, pH 9.0) respectively. After incubating the plate for 4 h at 37 °C the supernatants (50  $\mu\text{L}$ ) were transferred into new wells and measured using an automated fluorescence scanner (Titertek Fluoroskan II, Germany). The cytotoxicity was determined according to the equation described in Example 1.

- 13 -

## Results

Inhibition of endogenous MIA synthesis by specific phosphorothioate antisense oligonucleotides in human, MIA-secreting melanoma cell lines (GI and HW) was enhanced by up to 20 %autologous LAK cytotoxicity compared to untreated MIA-producing melanoma cell lines.

Active sequences inhibiting MIA expression were

MIA-2841-W	GTC AGG AAT CGG CAG	(Seq. ID No 1)
MIA-1278-W	CTT GGA GAA GAC ATA C	(Seq. ID No 2)
MIA-2842-W	TGC CTC CCC AGA AG	(Seq. ID No 3)

Less active or inactive sequences inhibiting MIA expression were

MIA-2843-N	CAC TGG CAG TAG AAA TC	(Seq. ID No 4)
MIA-2844-N	GCT CAC TGG CAG TAG	(Seq. ID No 5)
MIA-0202-N	ATG GTC AGG AAT CG	(Seq. ID No 6)
MIA-1277-N	GAA TGG TCA GGA ATC G	(Seq. ID No 7)
MIA-2328-N	CAT CGT GGA CTG TG	(Seq. ID No 8)

### Example 3

Furthermore, peptides inhibiting MIA activity in MIA-secreting melanoma cells also enhanced autologous LAK activity by up to 30%.

### Example 4

Inhibition of endogenous MIA synthesis by specific phosphorothioate antisense oligonucleotides in human, MIA-secreting melanoma as well as breast cancer cell lines strongly reduced their migration activity, as well as increasing their adhesion to matrices, both showing a strong inhibitory effect of MIA inhibitors on tumor invasion and metastasis.

### Example 5

Inhibition of endogenous MIA synthesis by specific phosphorothioate antisense oligonucleotides in human, MIA-secreting melanoma cell lines (GI and HW) in combination with application of the cytokines IL-12, IL-4, IL-18 and/or antisense oligonucleotides specific for TGF- $\beta$  increased the autologous LAK cytotoxicity even further compared to inhibition of endogenous MIA synthesis by specific phosphorothioate antisense oligonucleotides alone in MIA-producing tumor cell lines.

#### Example 6

Inhibition of endogenous MIA synthesis by a transfecting vector expressing the antisense sequence (SEQ. ID No 9):

GGCAGGGCCAGCGGTAGGCTGAGCTCACTGGCAGTAGAAATCCCATTGTCTGTCT  
TCACATCGACTTTGCCAGGTTTCAGGGTCTGGTCCTCTCGGACAATGCTACTGGGGA  
AATAGCCCAGGCGAGCAGCCAGATCTCCATAGTAATCTCCCTGAACGCTGCCTCCCC  
AGAAGAGCCGCCCACGGCCCTTCAGCTTGGAGAAGACATACACCACTTGGCCCCGG  
TGAATGGTCAGGAATCGGCAGTCGGGGGCCATGTAGTCCTGAAGGGCCACAGCCAT  
GGAGATAGGGTGGCTGCACTCCTGGTCCGCACACAGCTTCCGGTCAGCCAGCTTGG  
GCATAGGACCACCCCTGACACCAGGTCCGGAGAAGGCAGACAGCAAGATGATGACA  
CCAAGGCACACCAGGGACCGGGCCATCGTGGACTGTGAGCAAGAGAGTGAGCAAG  
GGGGTGCTGG

or parts of this sequence in human MIA-secreting melanoma as well as breast cancer cell lines strongly reduced their tumor invasion and metastasis in scid-mice and nude mice.

#### Example 7

Inhibition of tumor invasion and metastasis was increased by a combination of inhibitors of MIA with inhibitors of VEGF or TGF- $\beta$ .



Claims

1. A method for stimulating immune cells and/or the immune system, and/or reducing invasion and/or metastasis of tumor cells by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" MIA.
2. The method according to claim 1, wherein the inhibition of the expression and/or functional activity of MIA is achieved by using at least one nucleic acid molecule or derivative thereof,
3. The method according to claim 2 wherein the at least one nucleic acid molecule is an oligonucleotide, an antisense nucleic acid and/or a ribozyme.
4. The method according to claim 1, wherein the inhibition of the synthesis and/or function of MIA is achieved using a molecule comprising the antisense sequences SEQ ID No. 1 to 3 or SEQ ID No. 10 to 39 or parts of the sequences having at least 8 nucleotides.
5. The method according to claim 3 wherein the antisense and/or ribozyme molecule is derived by synthesising a sequence wholly or partially complementary to MIA mRNA and testing for inhibitory activity of MIA.
6. The method according to claim 3 wherein the antisense and/or ribozyme molecule is integrated into a DNA delivery system comprising viral and/or non-viral vectors together with lipids selected from the group of anionic lipids, cationic lipids, non-cationic lipids and mixtures thereof.
7. The method according to claim 3 wherein the antisense and/or ribozyme molecule is modified at one or more of the sugar moieties, the bases and/or the internucleotide linkages and/or by coupling the antisense and/or ribozyme molecule to an enhancer of uptake and/or inhibitory activity.

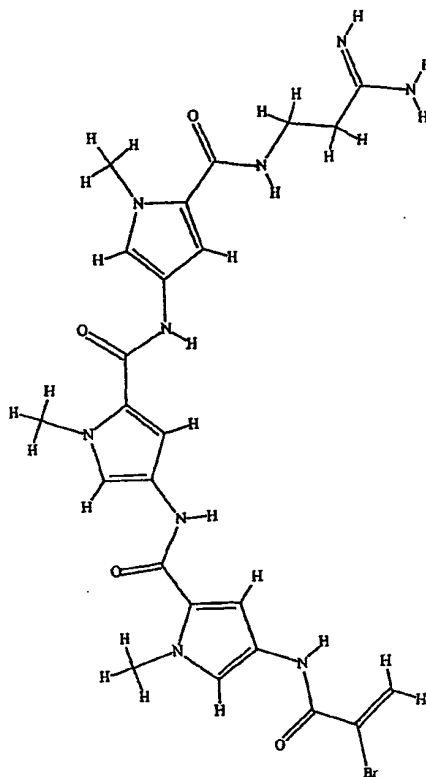
- 16 -

8. The method according to claim 1 wherein the inhibition of the expression and/or functional activity of MIA is achieved using peptides and/or proteins.
9. The method of claim 8 wherein the peptides and/or proteins comprise the sequences SEQ ID No. 40 to 63 and analogs or derivatives thereof.
10. The method according to claim 8 wherein the peptide and/or protein is derived by screening an expression library and testing the expression products for inhibitory activity of MIA.
11. The method according to claim 8 wherein the peptide and/or protein is derived by screening randomly synthesised peptides and/or proteins for inhibitory activity of MIA.
12. The method according to claim 1, wherein the inhibition of the expression and/or the function activity of MIA is achieved using an inhibitor of low molecular weight.
13. The method of claim 12 wherein the inhibitor of low molecular weight is selected from compounds having any one of the structures 1 to 492 of Fig. 1 to 42 or comprise any of these structures as substructures, or parts of the structures 1 to 492 comprising at least an aromatic system and an amid bond.
14. The method according to claim 12 wherein the inhibitor of low molecular weight is obtainable by combinatorial chemistry and testing the products for inhibitory activity of MIA.
15. The method according to claim 1, wherein the inhibition the expression and/or functional activity of MIA is achieved using DNA or RNA derivatives including aptamers and/or spiegelmers that bind to MIA.
16. The method according to claim 1 wherein the inhibition of MIA is achieved using antibodies or antibody fragments, such as F<sub>ab</sub>-fragments, single chain antibody or combinations thereof.

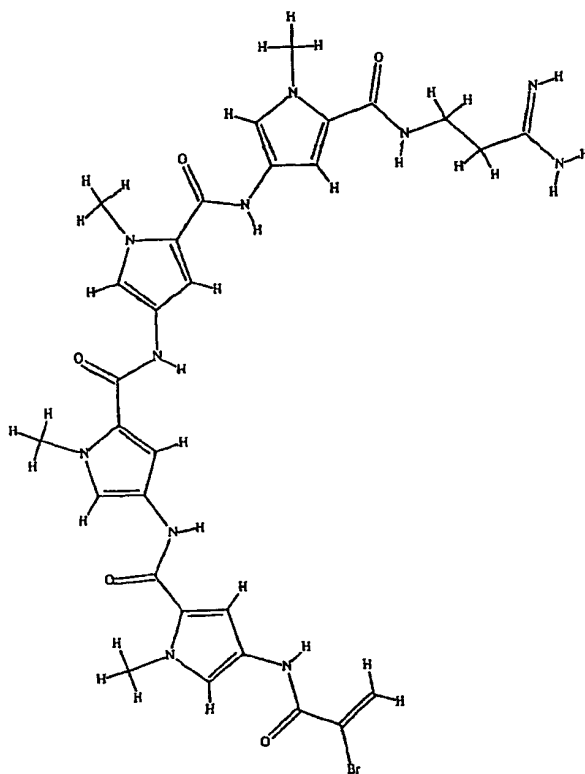
- 17 -

17. The method according to claim 16 wherein the antibody or antibody fragments, such as F<sub>ab</sub>-fragments, single chain antibody or combinations thereof are obtainable by screening antibody libraries and testing the expression products for inhibitory activity of MIA.
18. The method according to any of the claims 1 to 17 wherein additionally an immunostimulatory agent, such as cytokines and/or inhibitors of the expression and/or function of interleukin-10 and/or transforming growth factor beta (TGF- $\beta$ ) and/or Prostaglandin B2 and/or receptors for Prostaglandin E2 and/or inhibitors of VEGF.
19. A composition comprising a molecule or a combination of molecules for inhibiting the synthesis and/or function of MIA
20. A composition according to claim 19 wherein the molecule is selected from the oligonucleotides having any one of the SEQ. ID. No. 1 to 3 and SEQ ID No. 10 to 39 or parts of these sequences having at least 8 nucleotides.
21. A medicament comprising an inhibitor of the synthesis and/or function of MIA.
22. A medicament comprising an inhibitor of the synthesis and/or function of MIA combined with an immunostimulatory agent.
23. The use of the composition according to claim 19 for the preparation of a medicament for the prevention or the treatment of neoplasms, infections and/or immunosuppressive disorders.

1

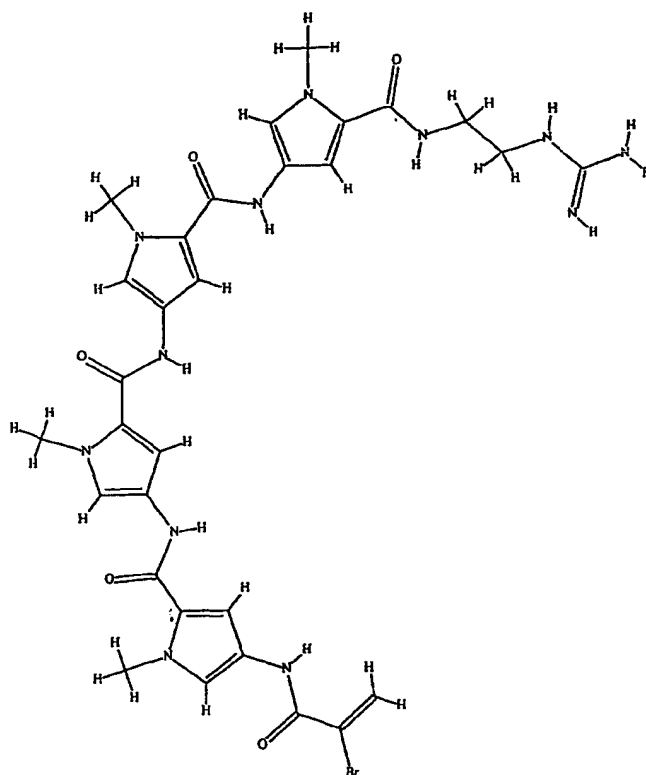


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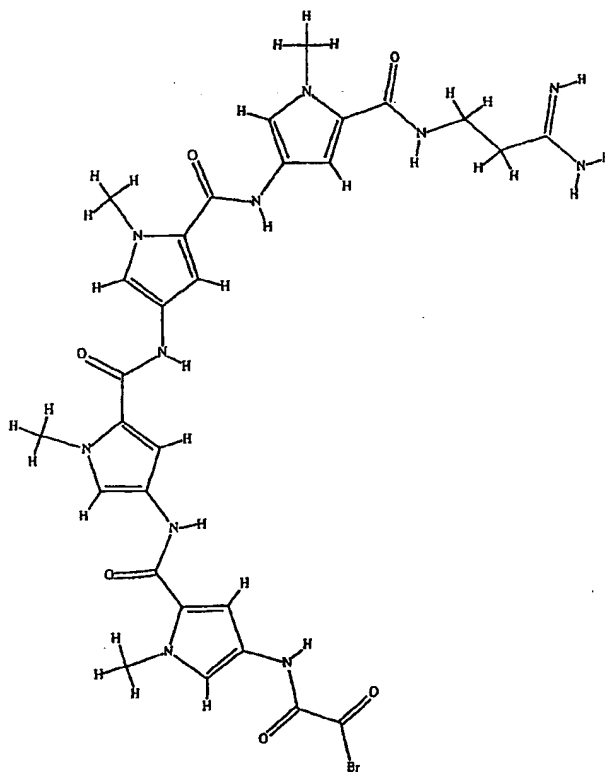


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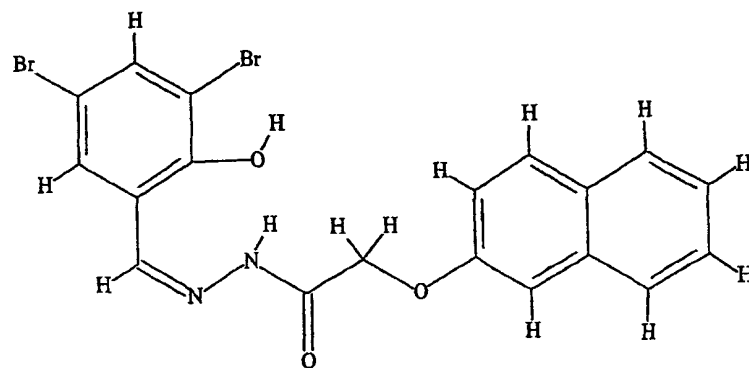
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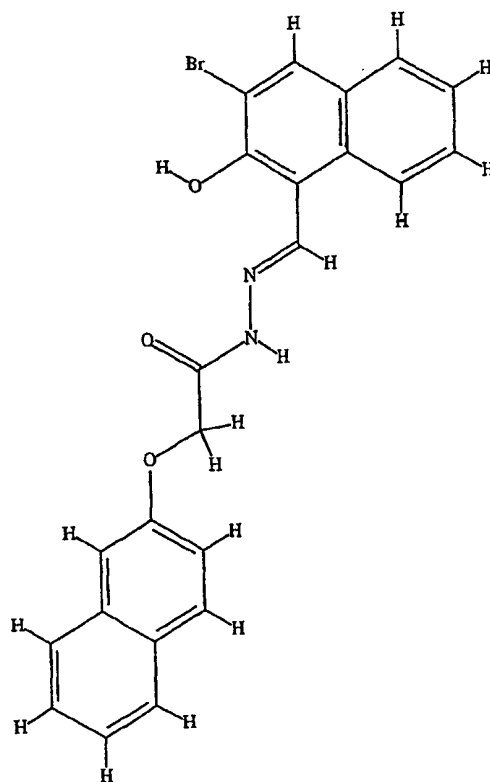
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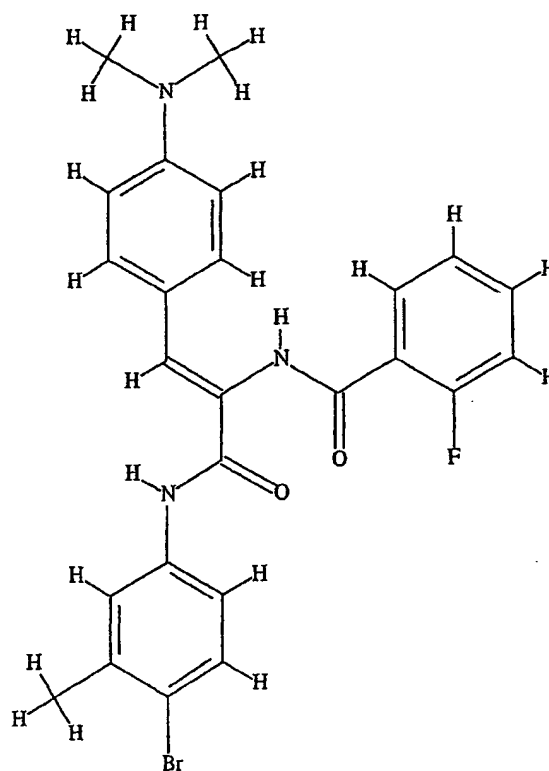
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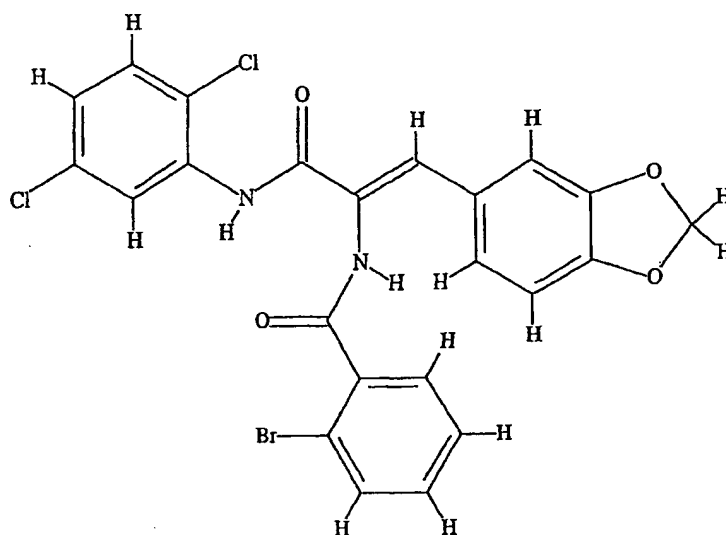
SUBSTITUTE SHEET (RULE 26)

4/248

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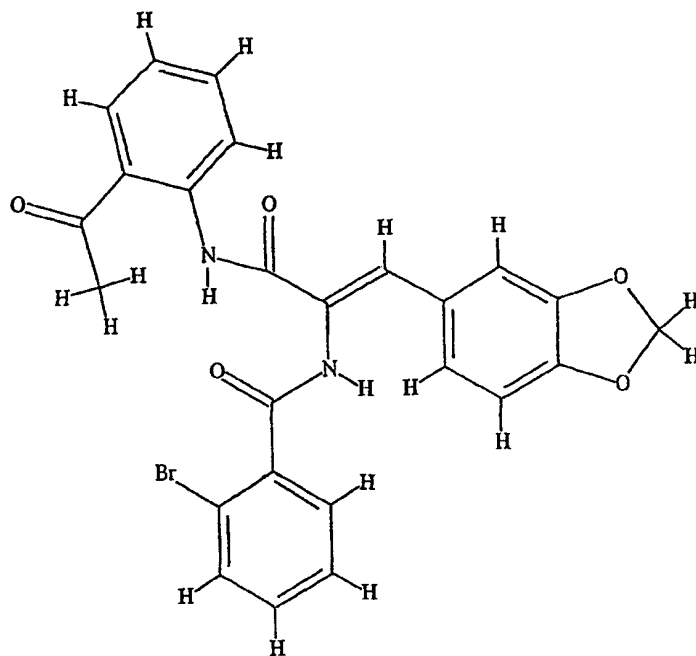


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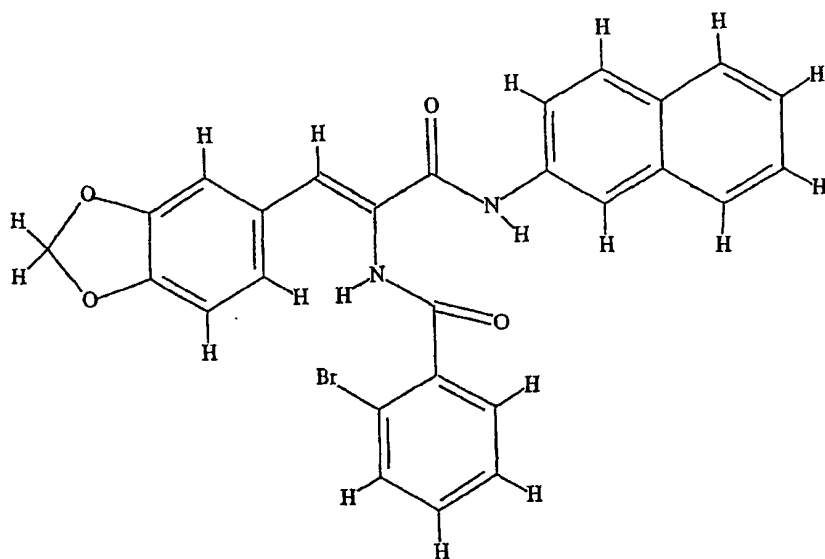


SUBSTITUTE SHEET (RULE 26)

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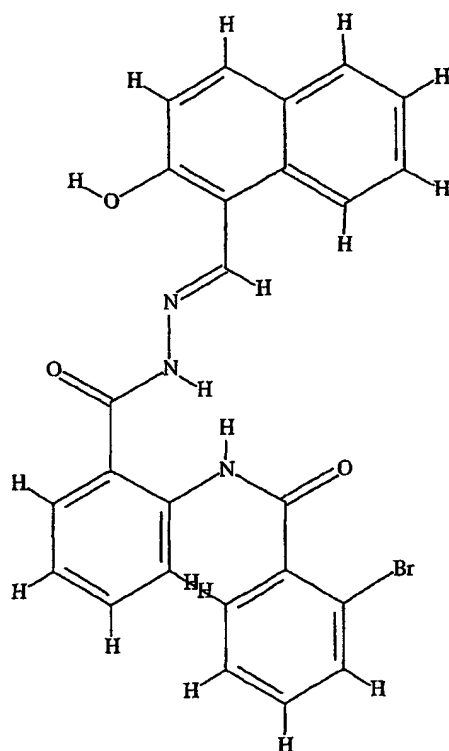


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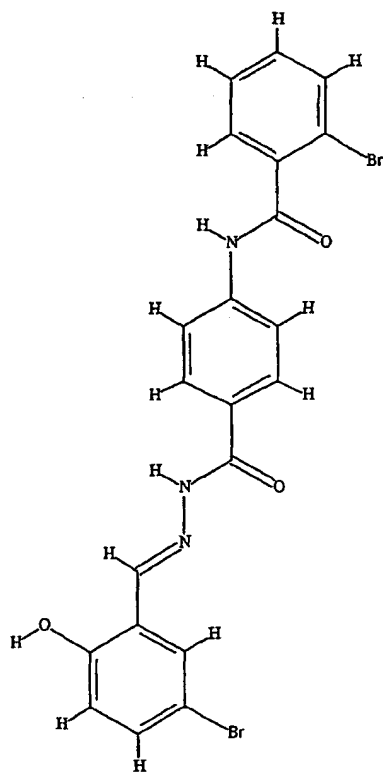


6/248

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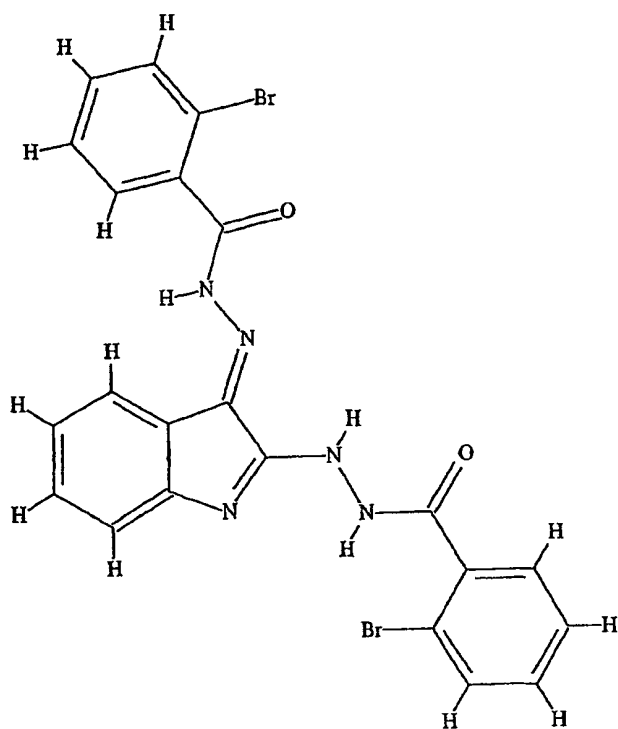
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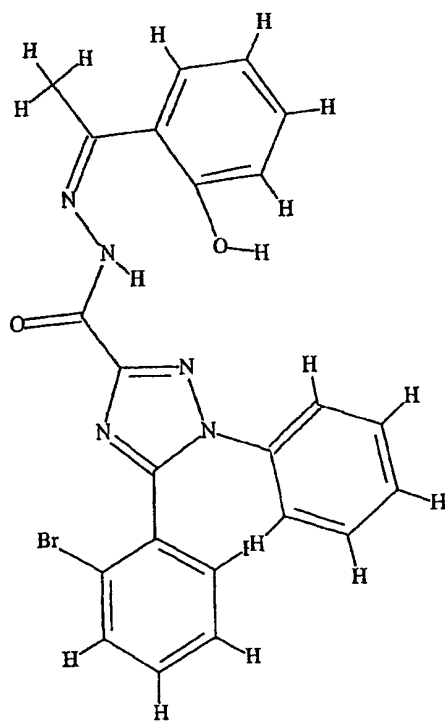
SUBSTITUTE SHEET (RULE 26)

7/248

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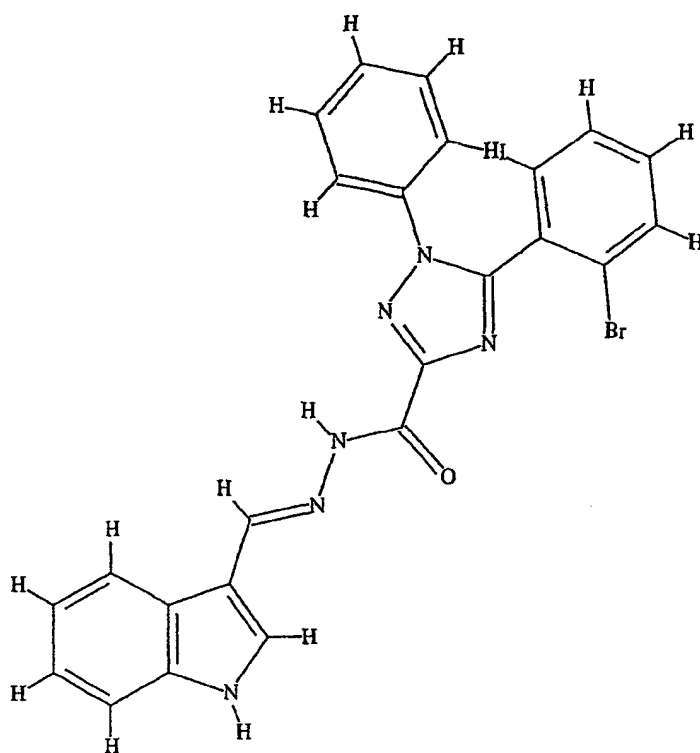
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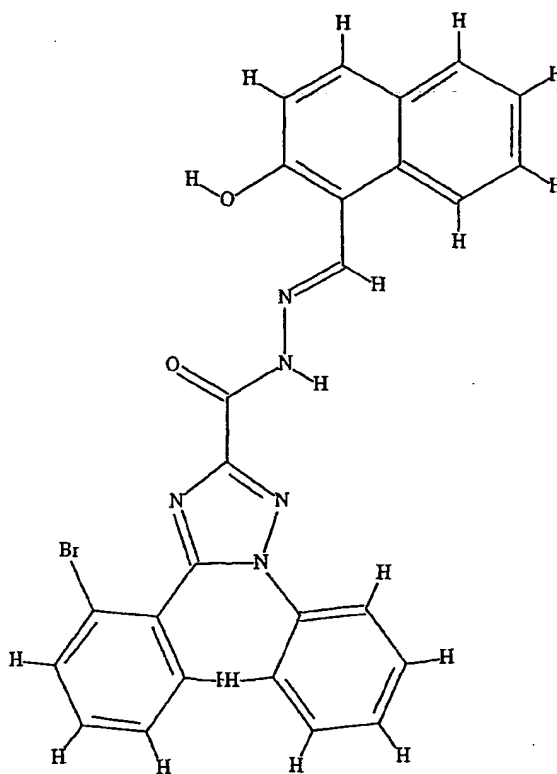
SUBSTITUTE SHEET (RULE 26)

8/248

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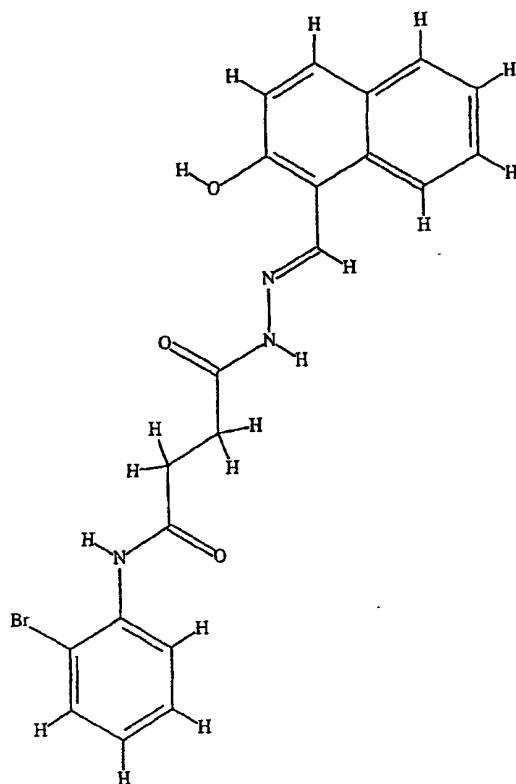
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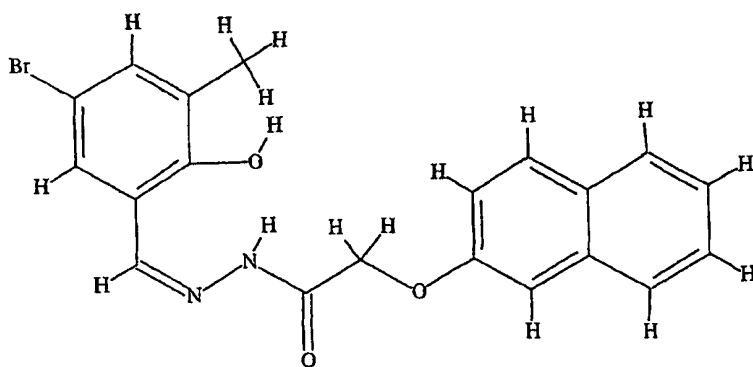
SUBSTITUTE SHEET (RULE 26)

9/248

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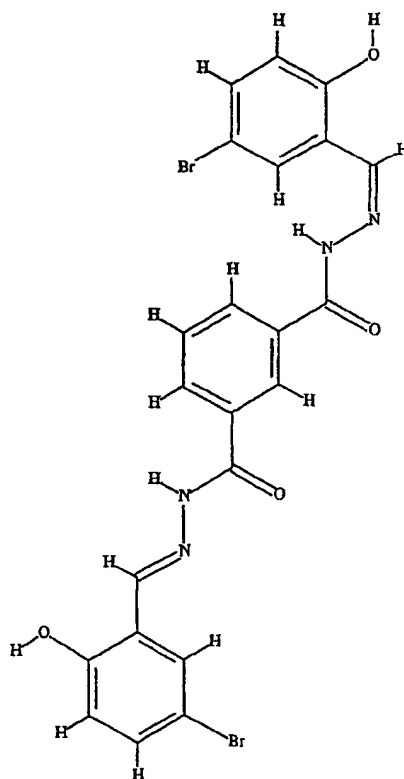
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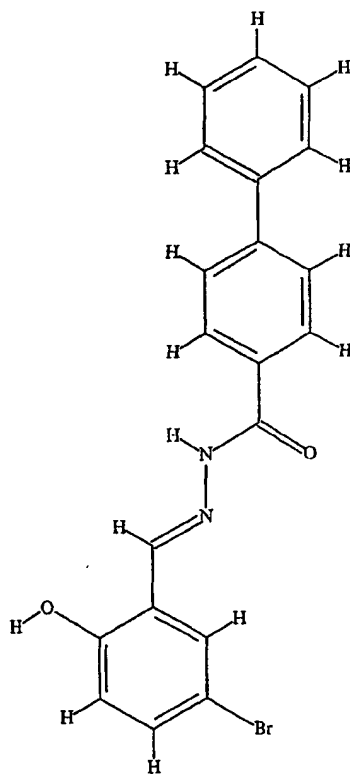
SUBSTITUTE SHEET (RULE 26)

10/248

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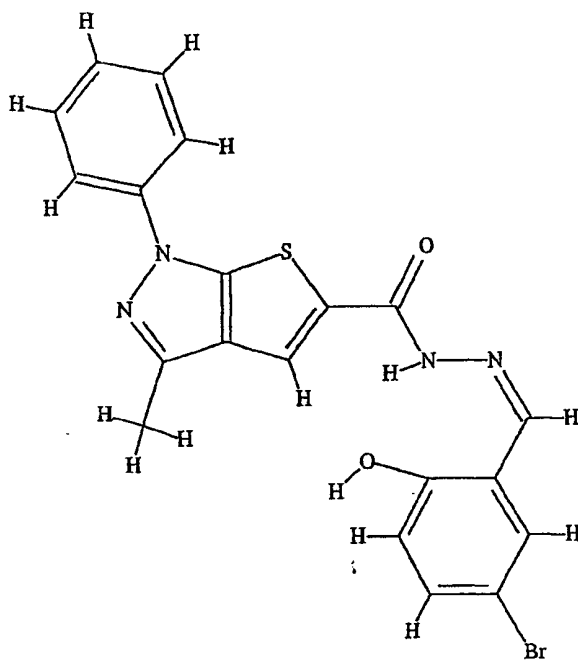
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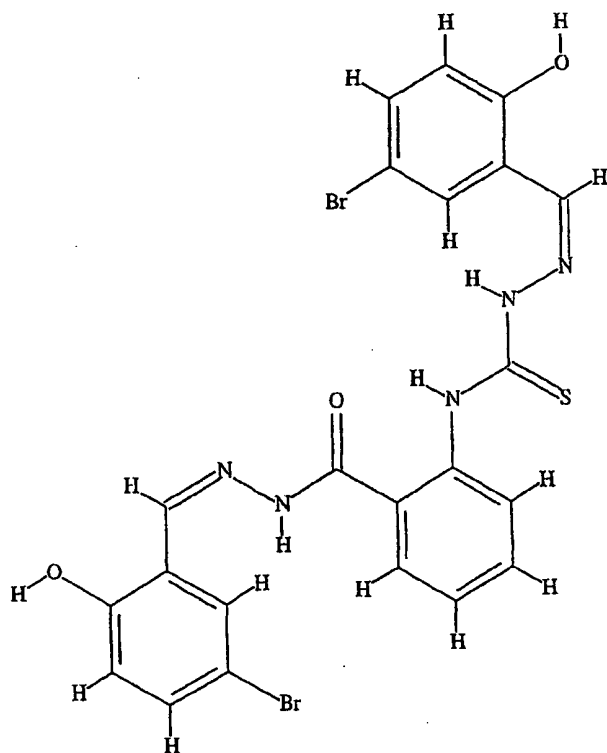
SUBSTITUTE SHEET (RULE 26)

11/248

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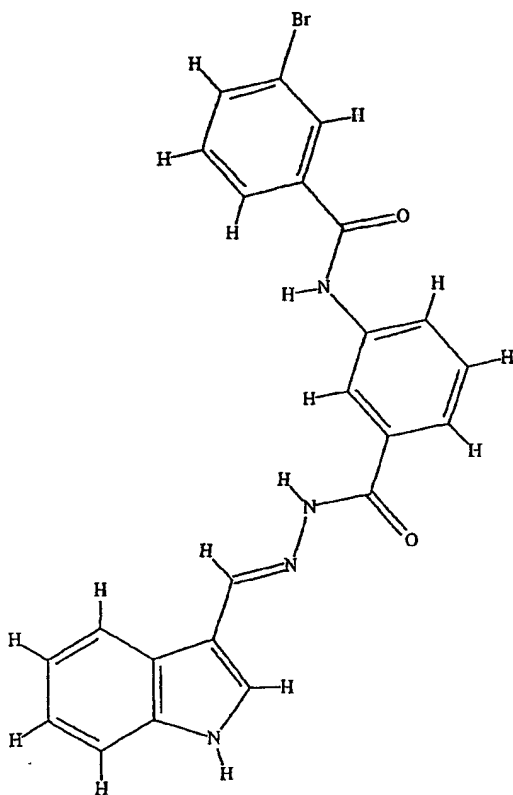
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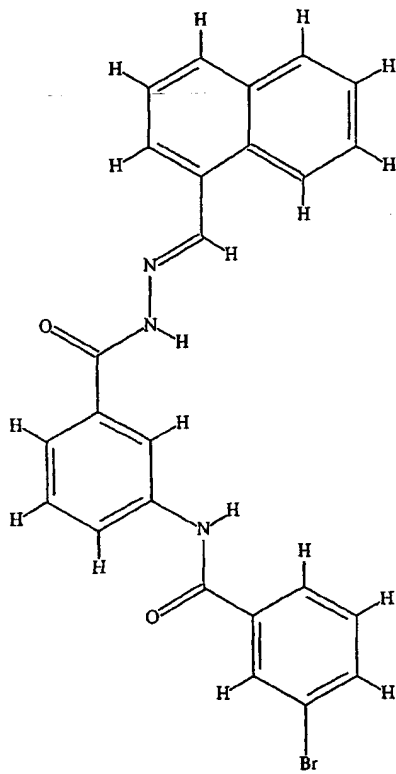
SUBSTITUTE SHEET (RULE 26)

12/248

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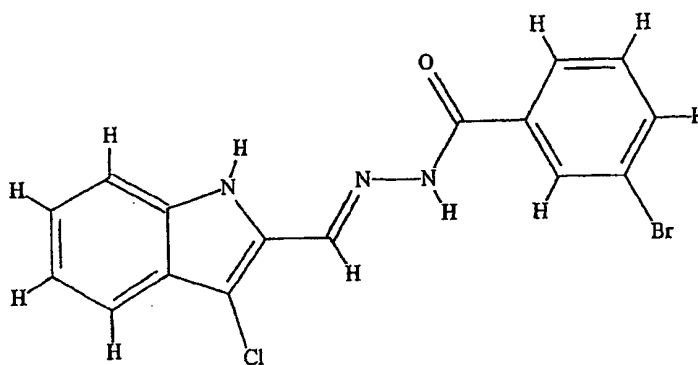


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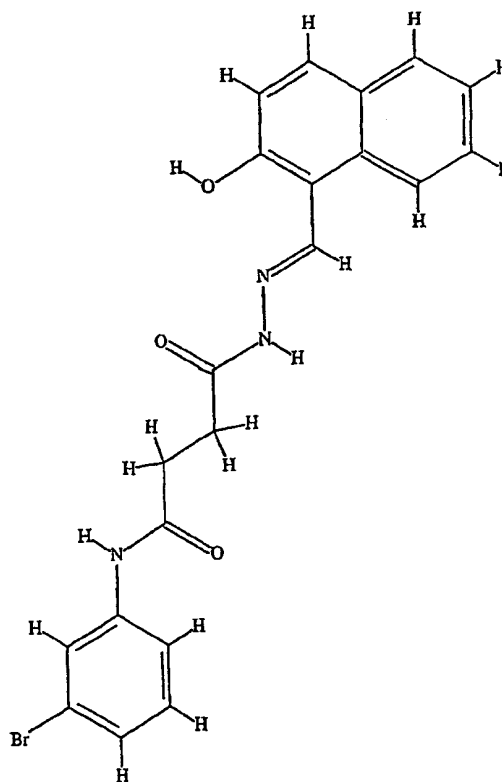


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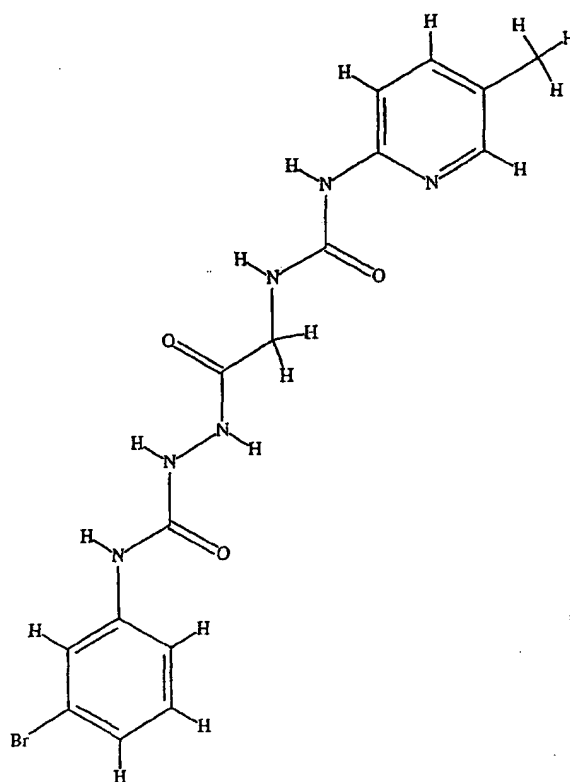


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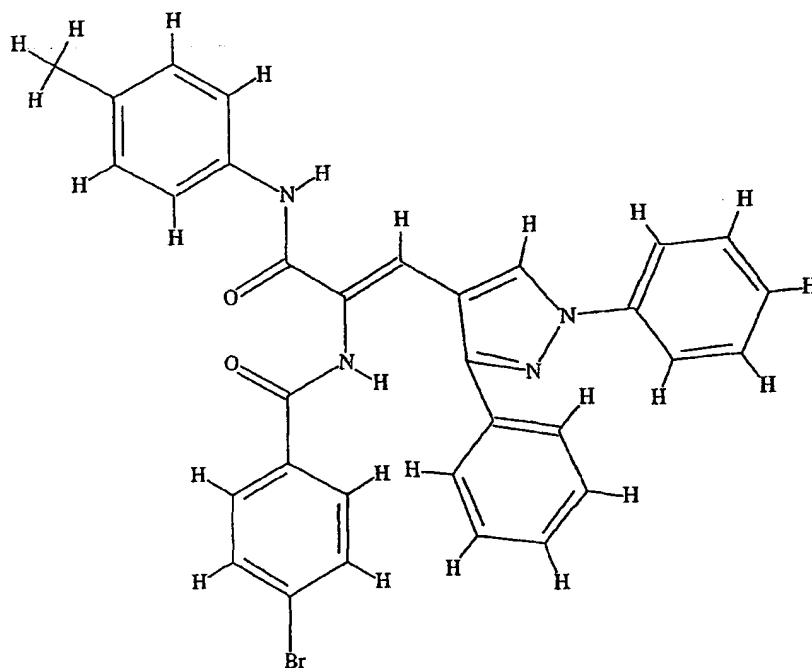


14/248

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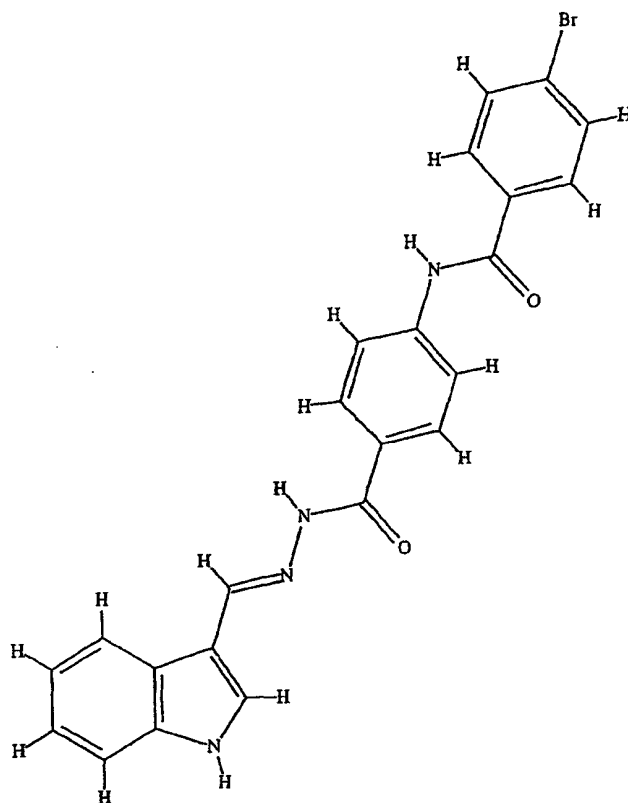


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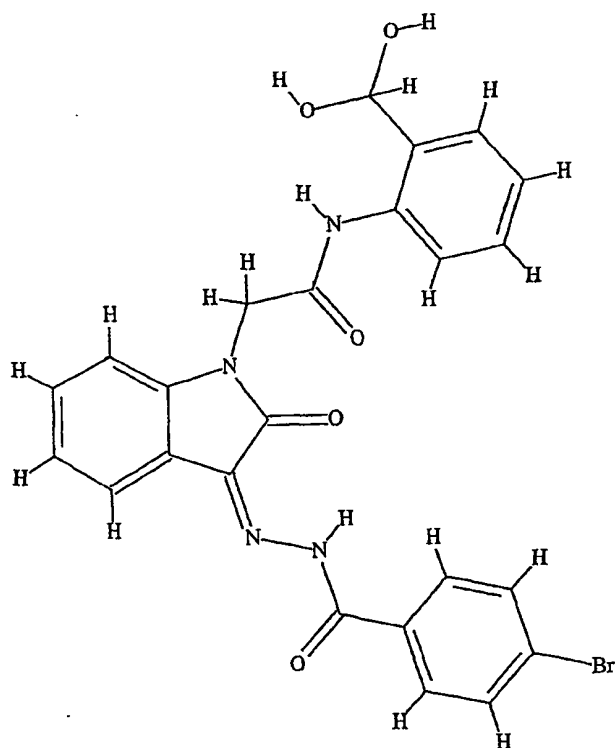


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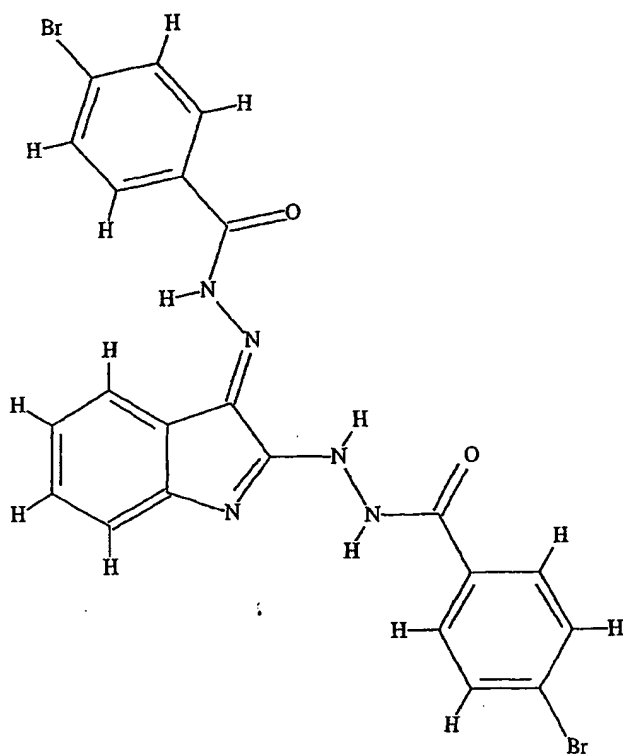
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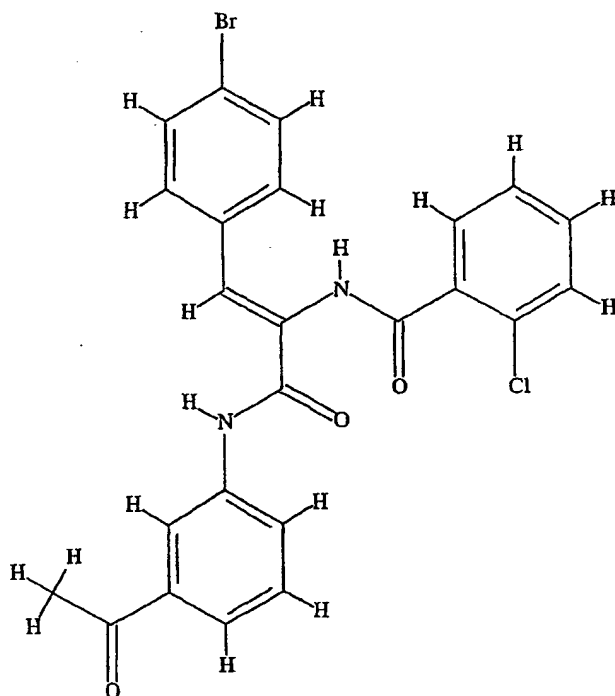
SUBSTITUTE SHEET (RULE 26)

16/248

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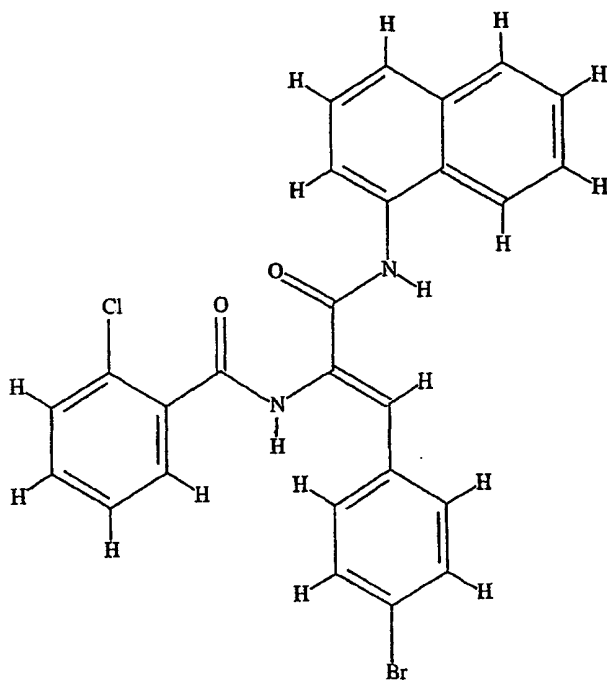


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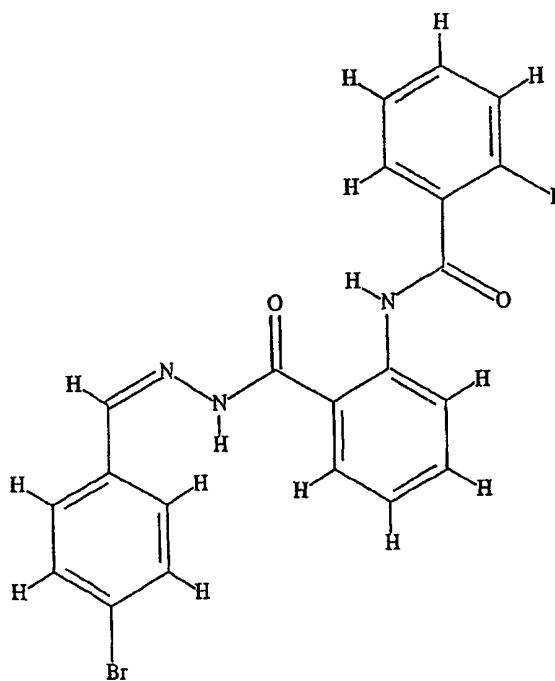


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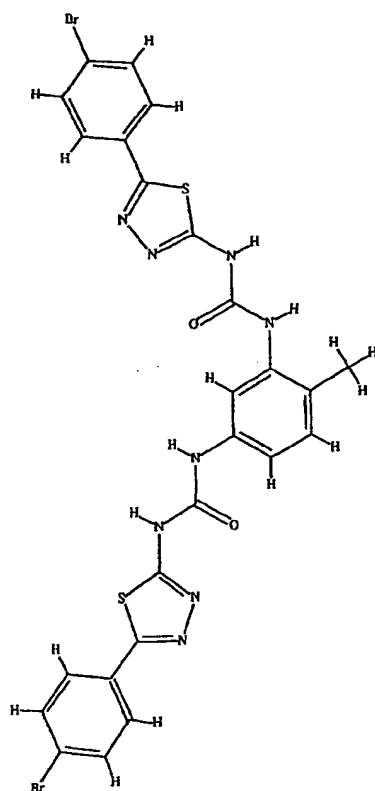


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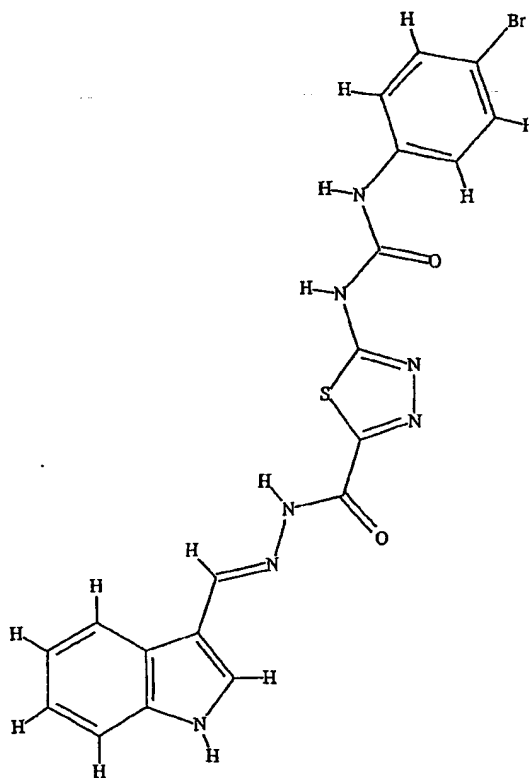


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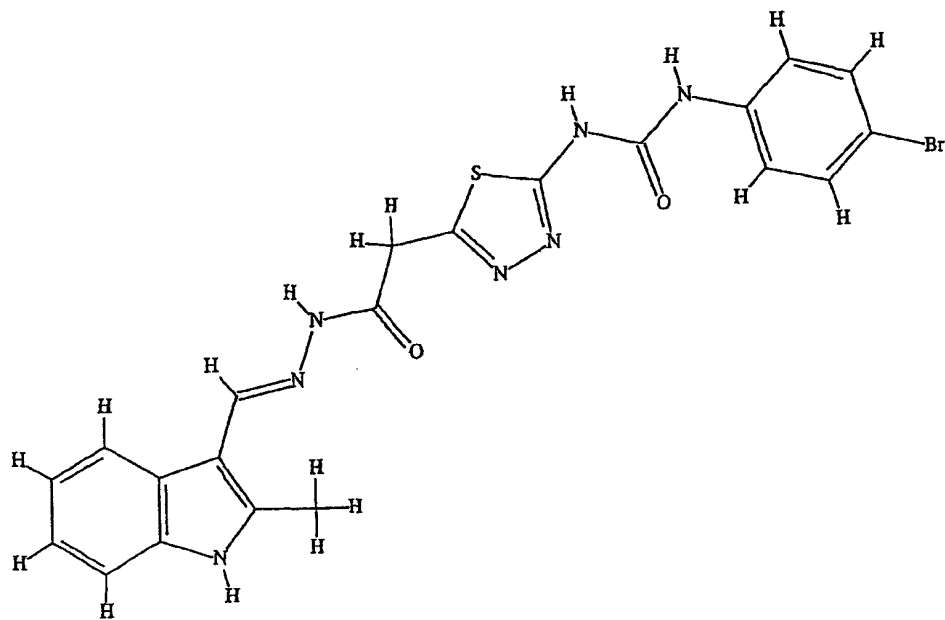
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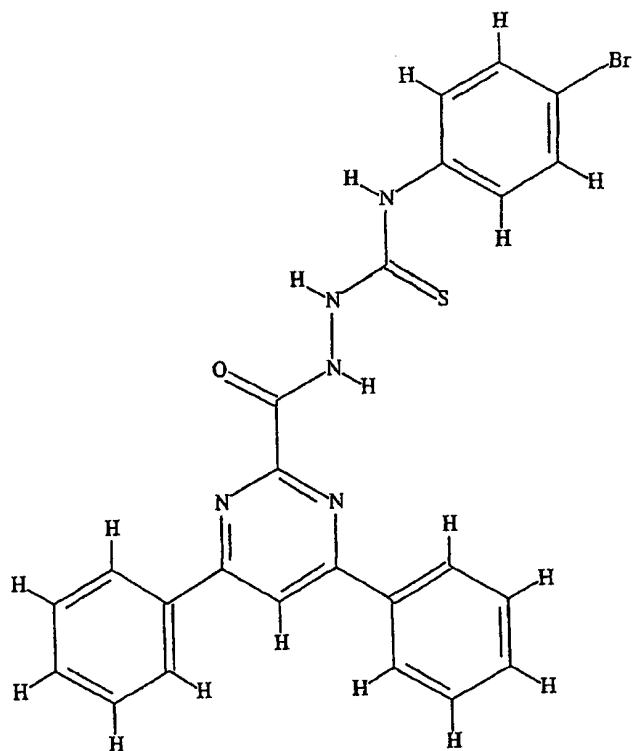
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SUBSTITUTE SHEET (RULE 26)



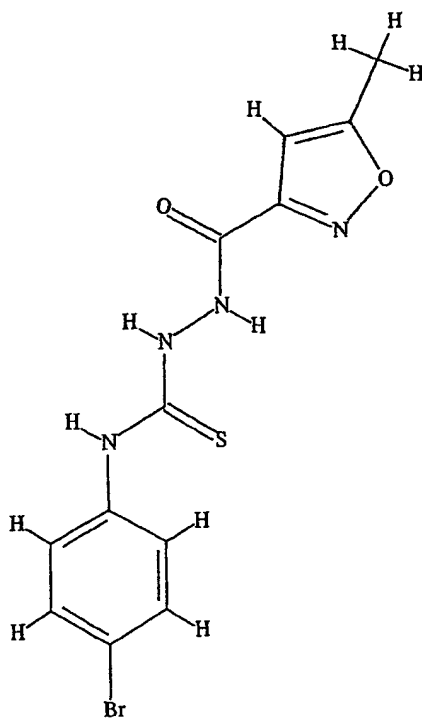
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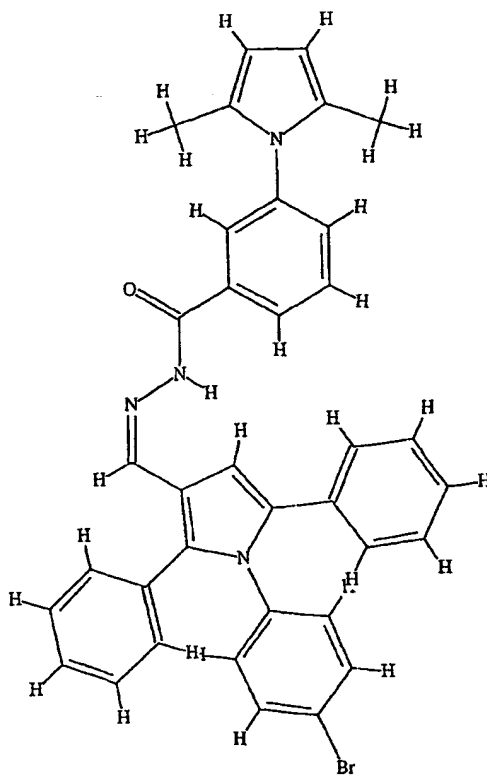
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20/248

39

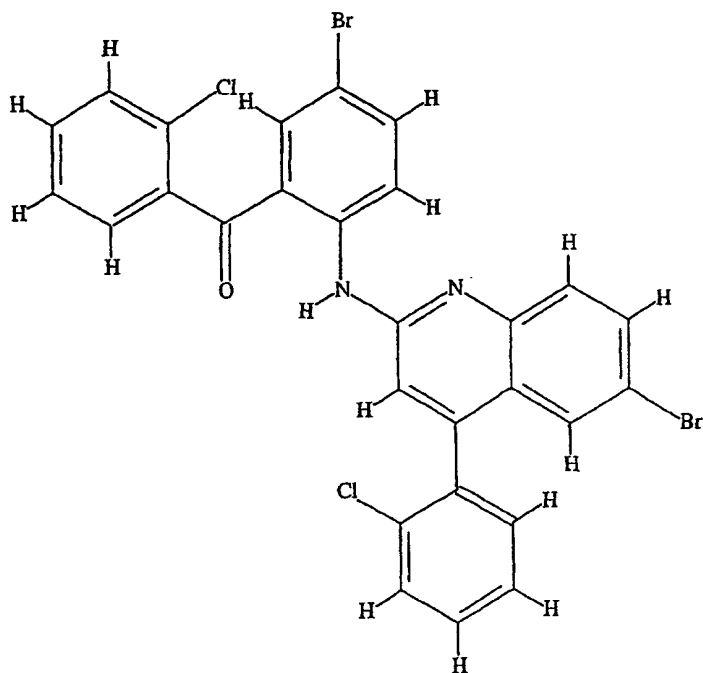


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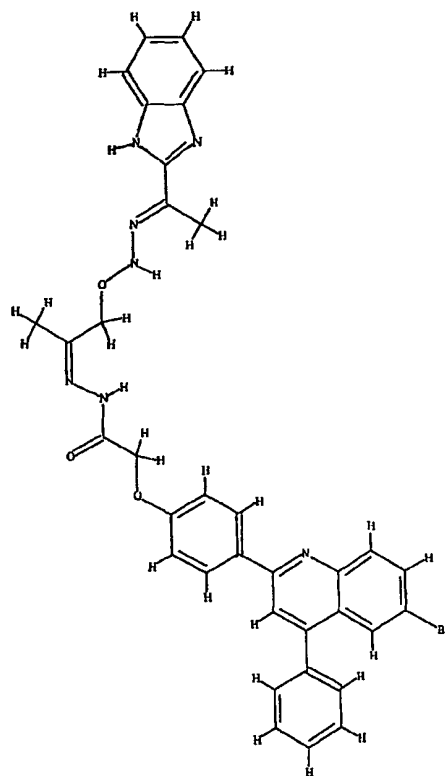


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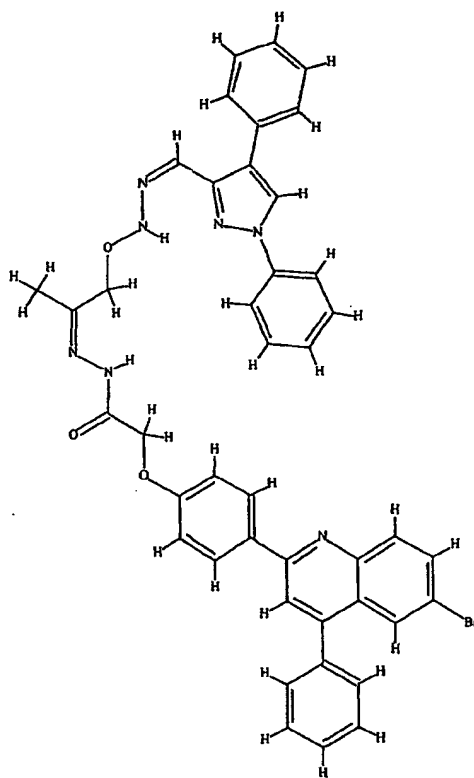


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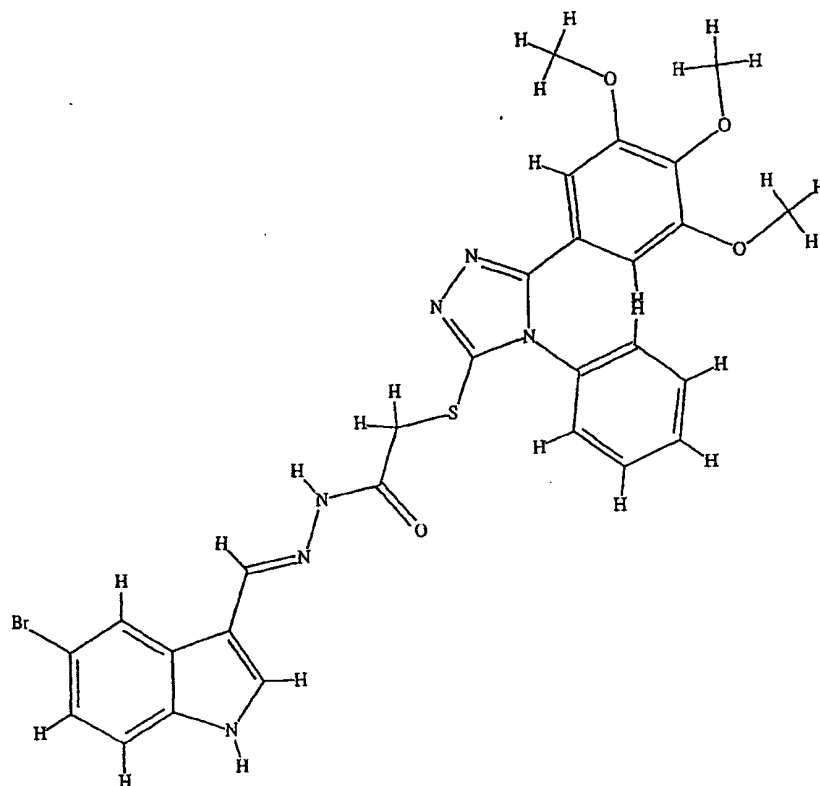
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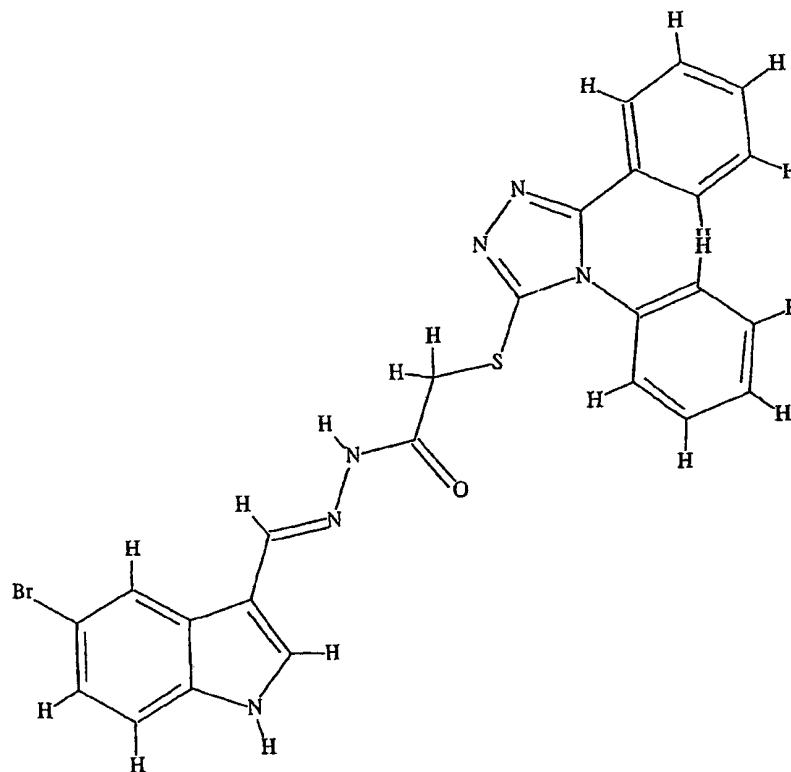
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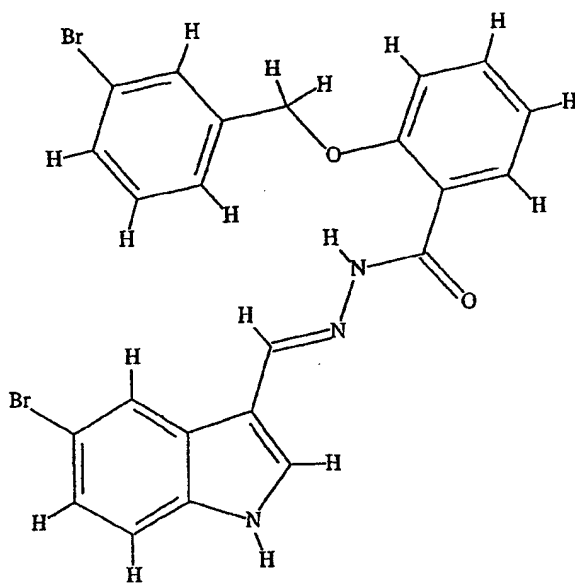


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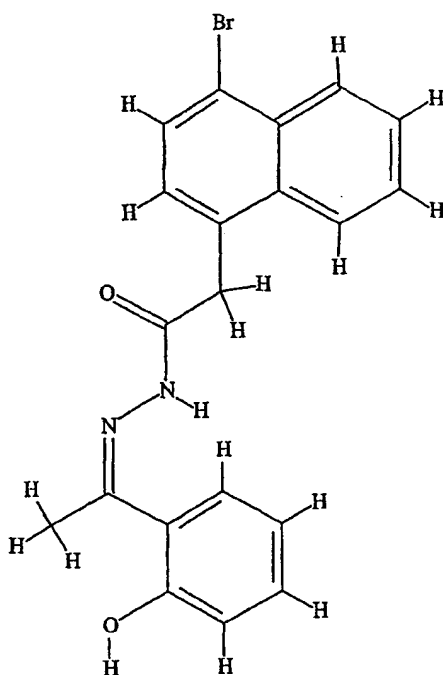


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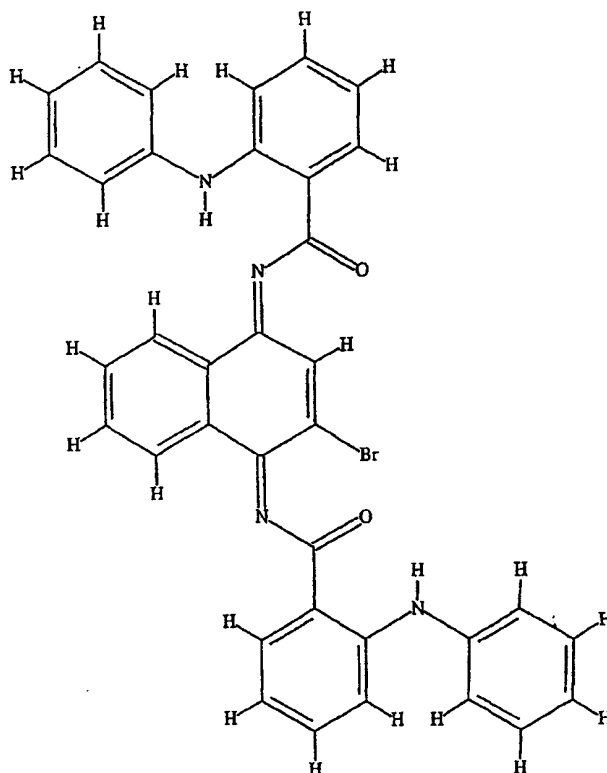


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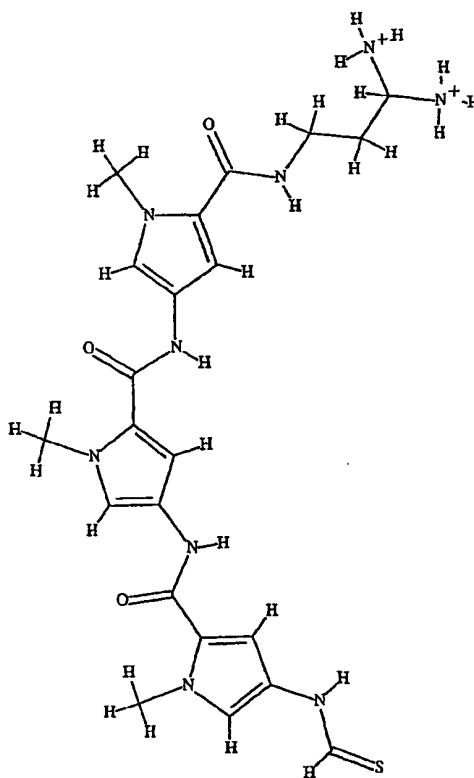


25/248

49



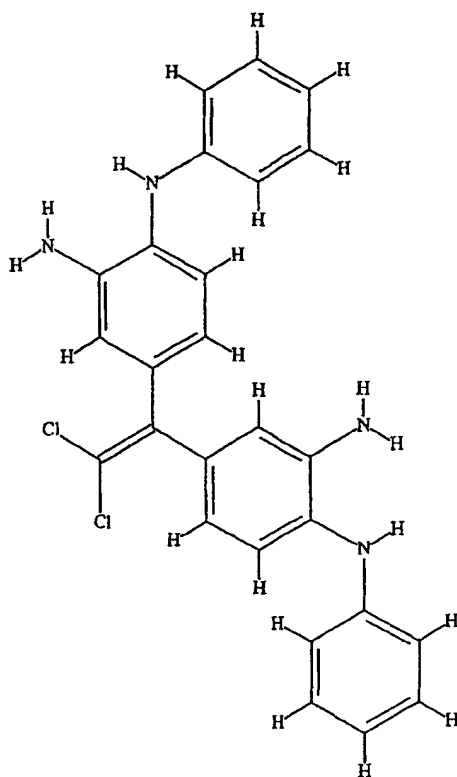
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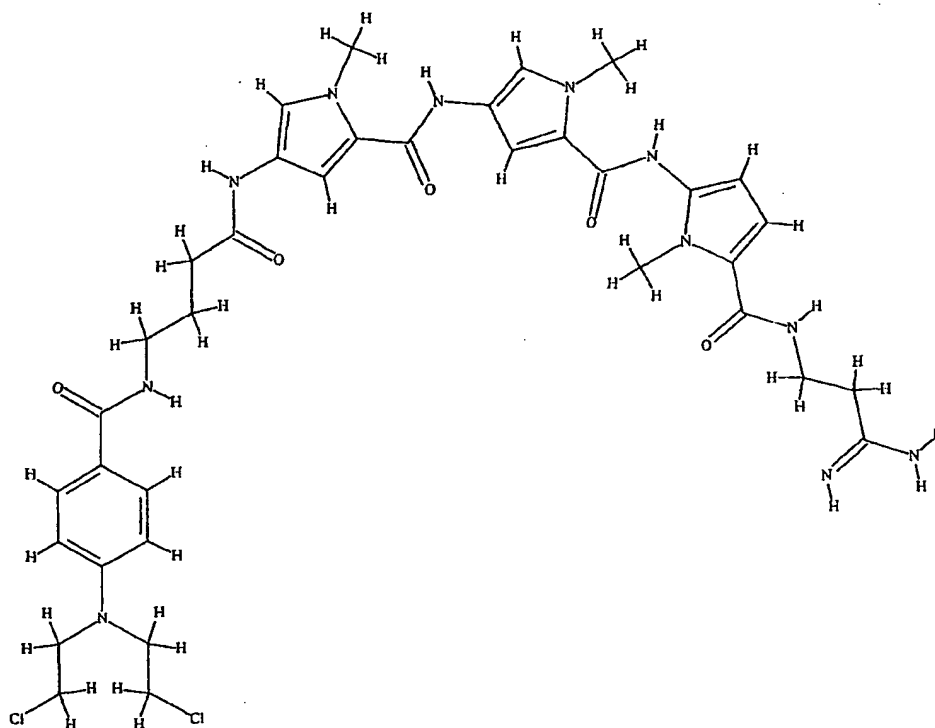
SUBSTITUTE SHEET (RULE 26)

26/248

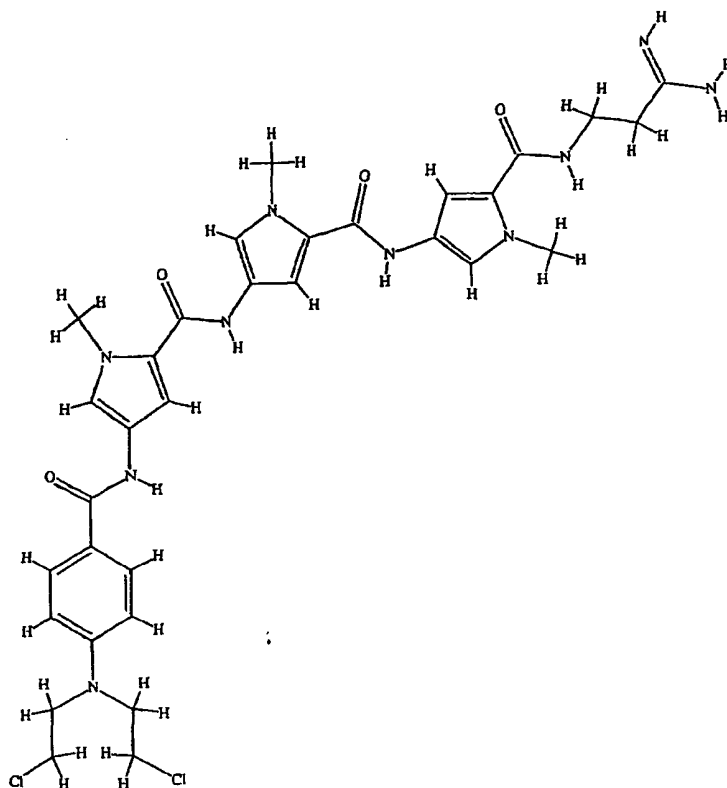
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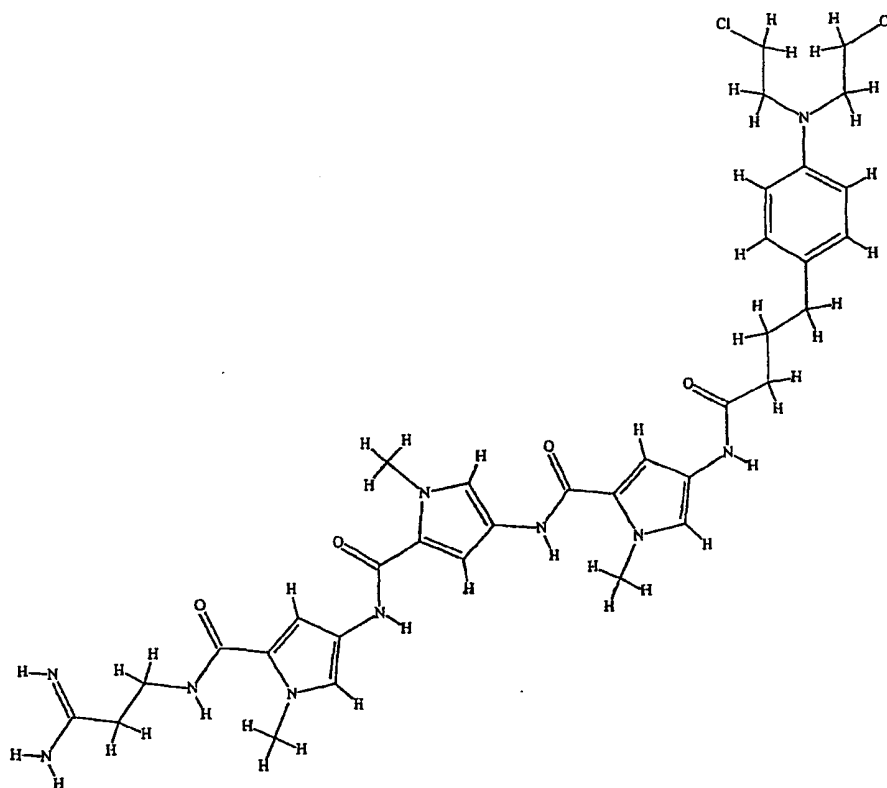
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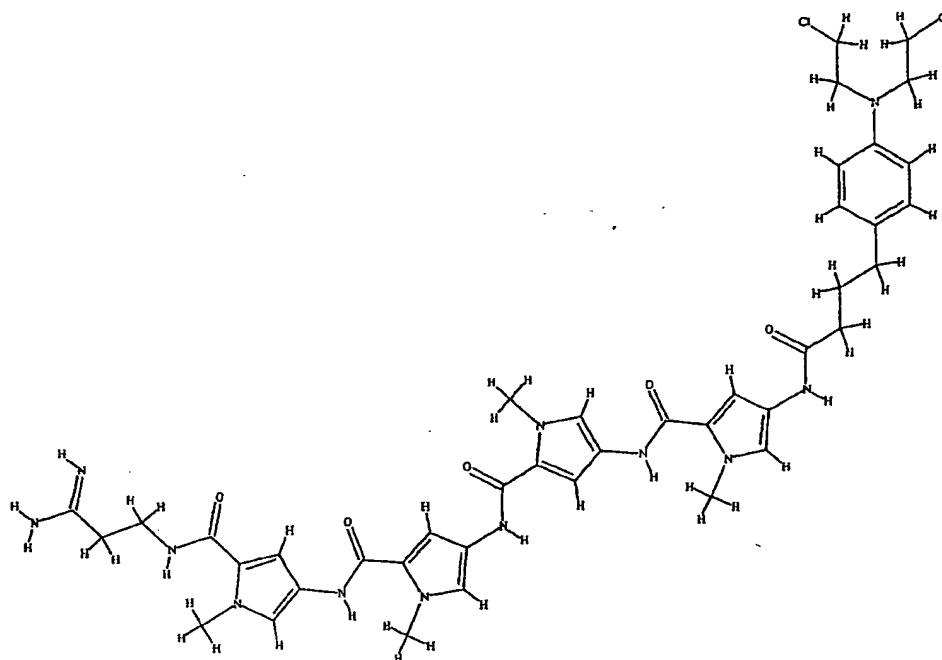


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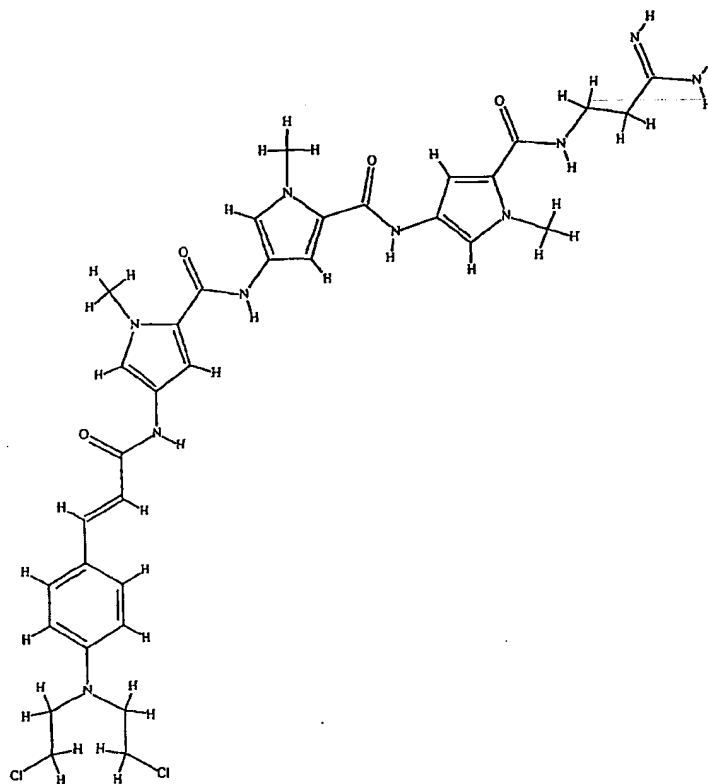


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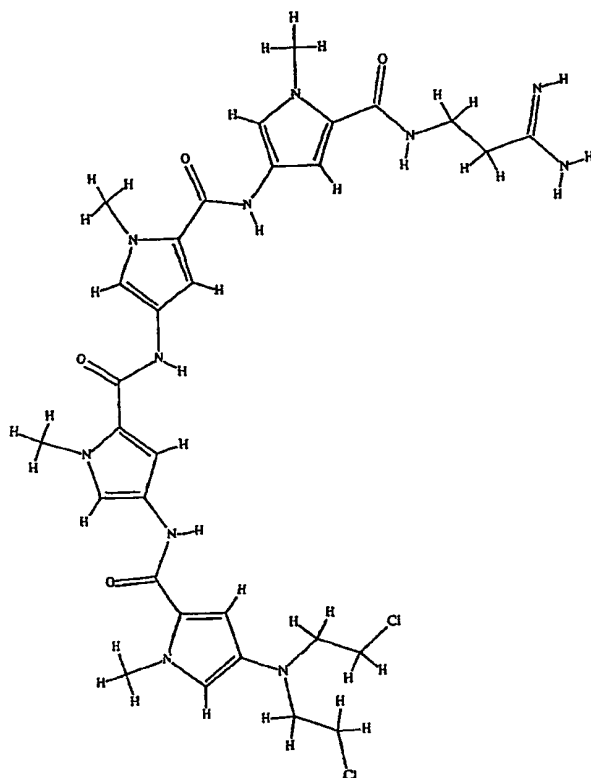


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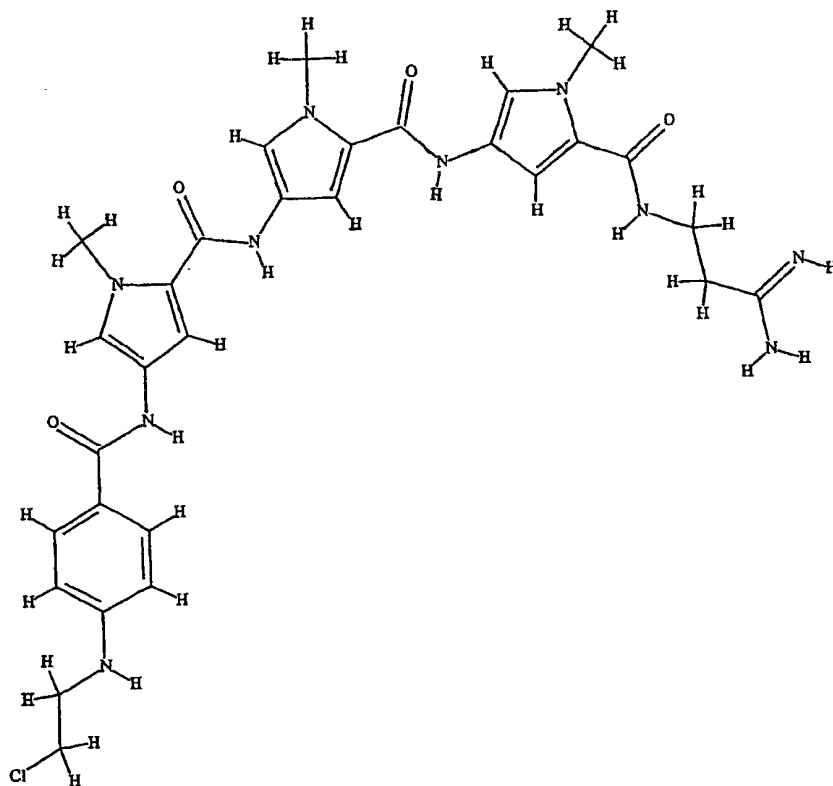


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57



58



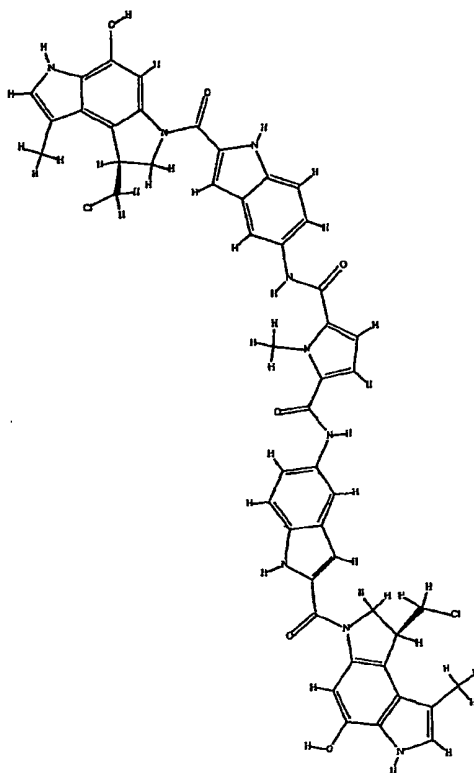
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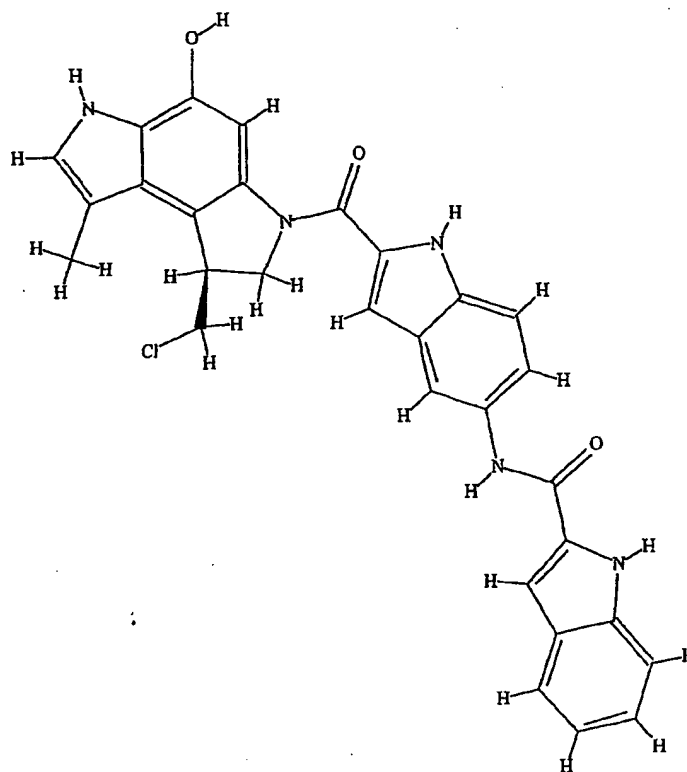
31/248

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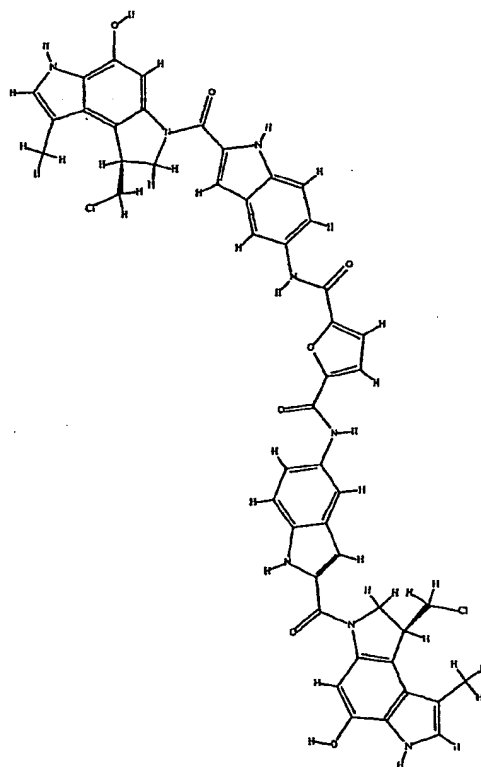


32/248

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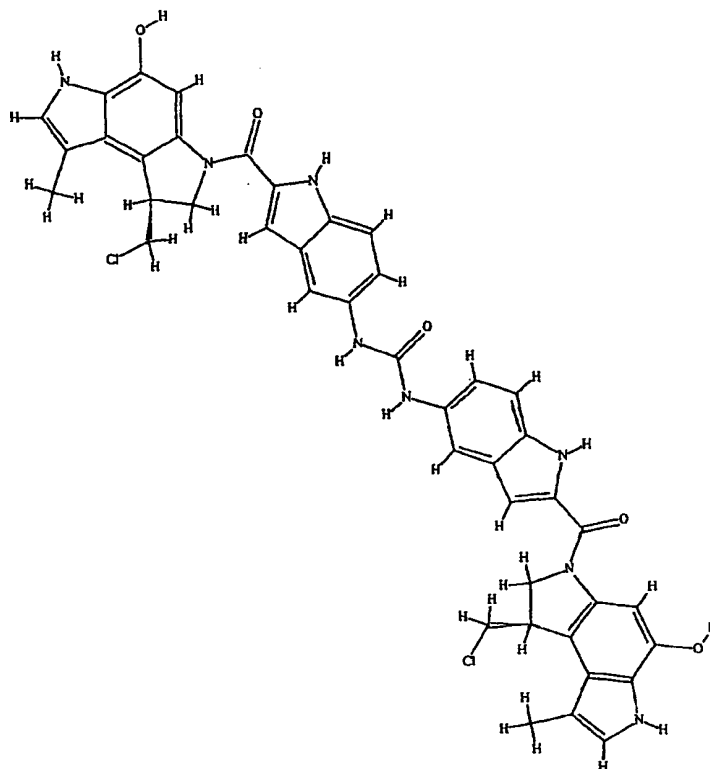
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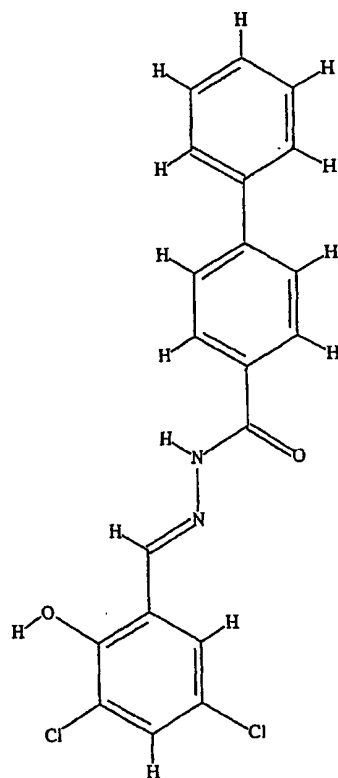
SUBSTITUTE SHEET (RULE 26)

33/248

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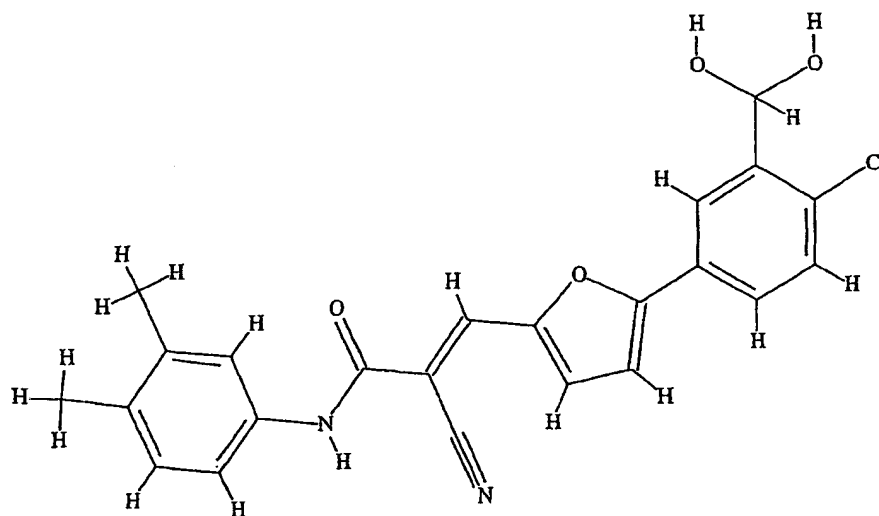


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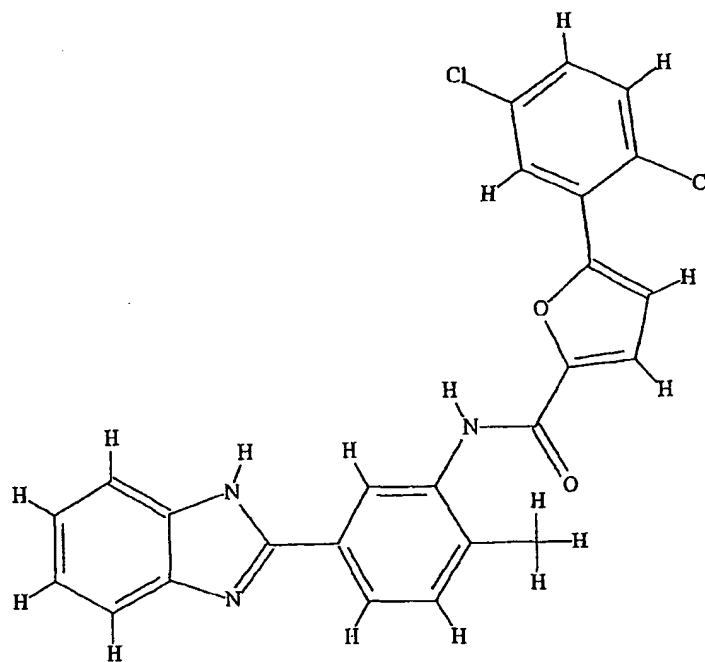


SUBSTITUTE SHEET (RULE 26)

67

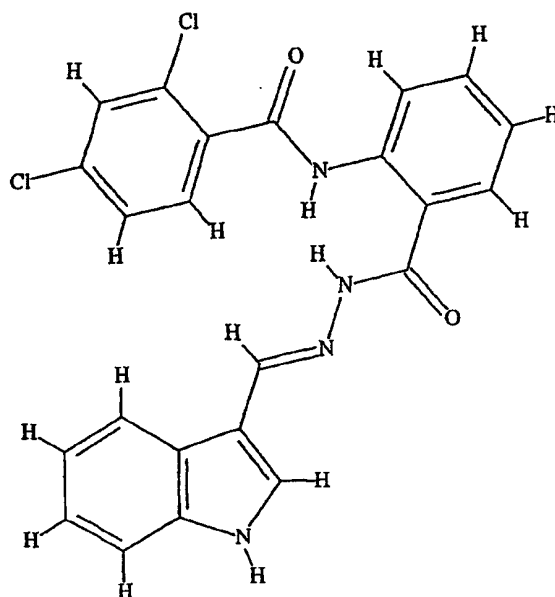


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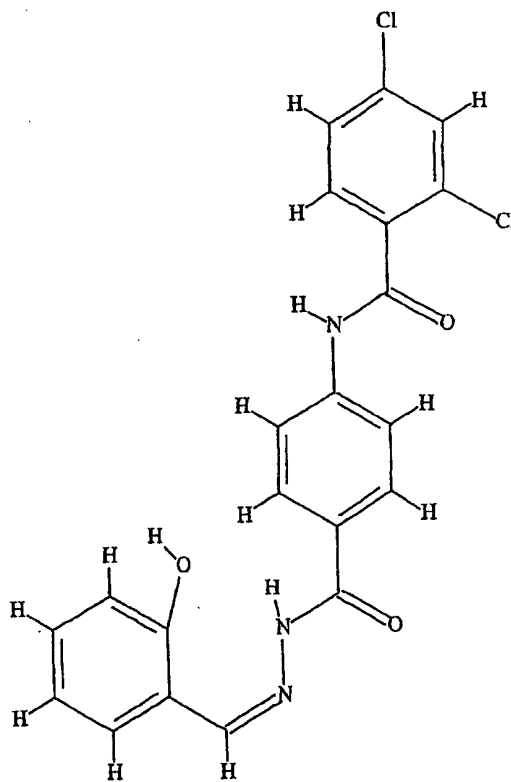


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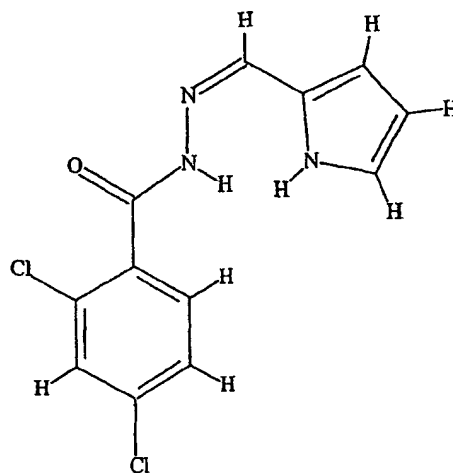


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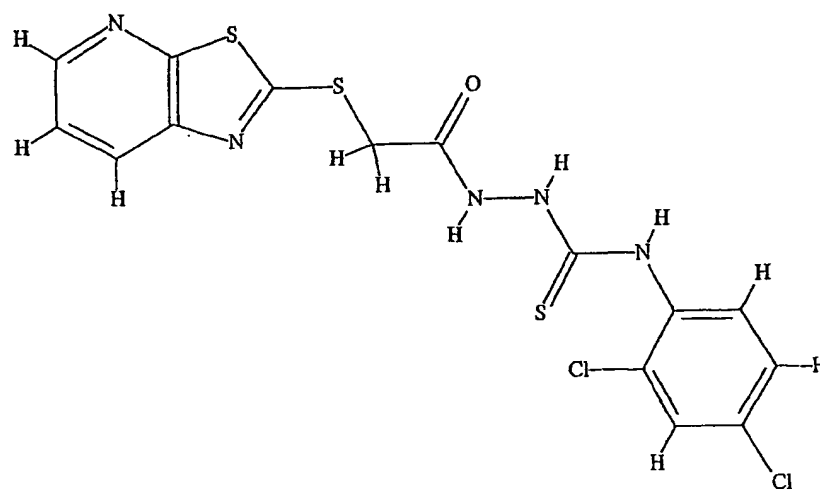


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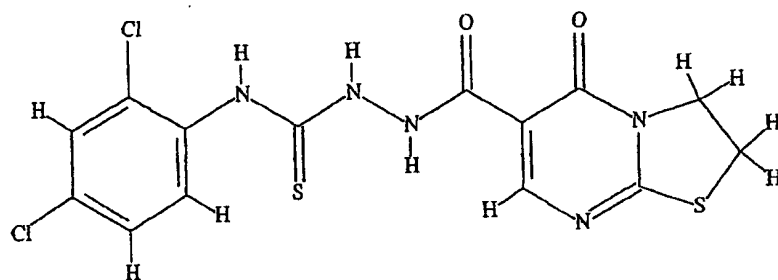


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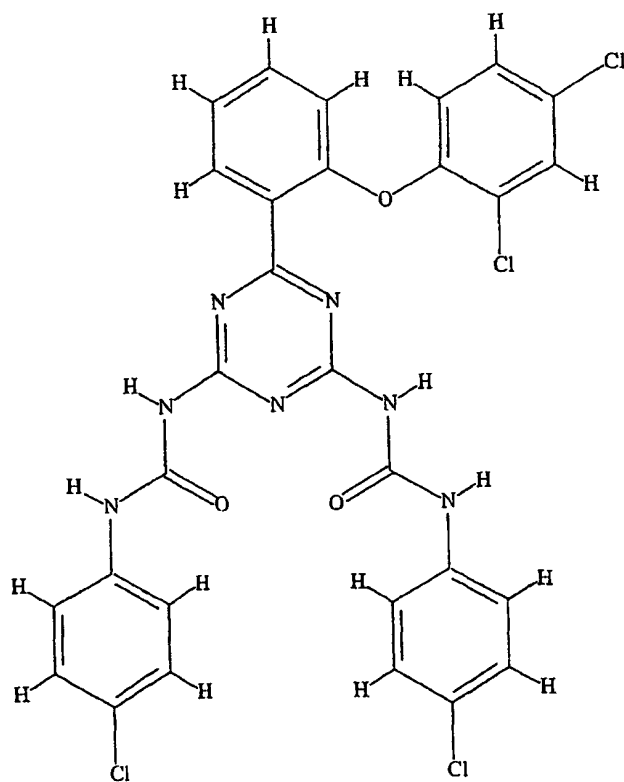


SUBSTITUTE SHEET (RULE 26)

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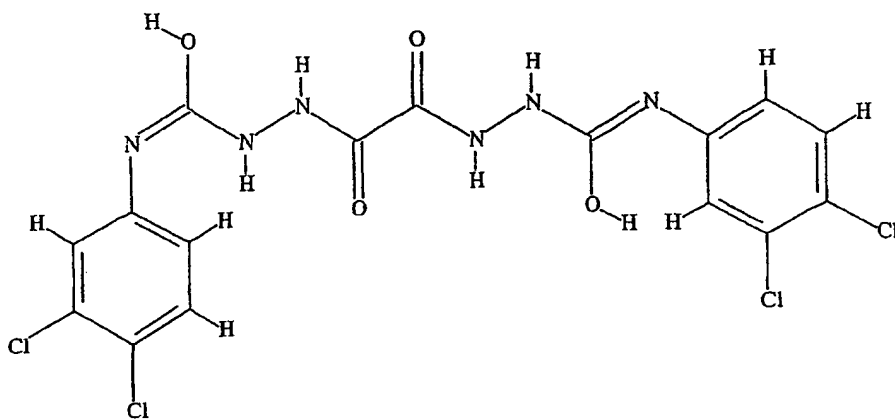
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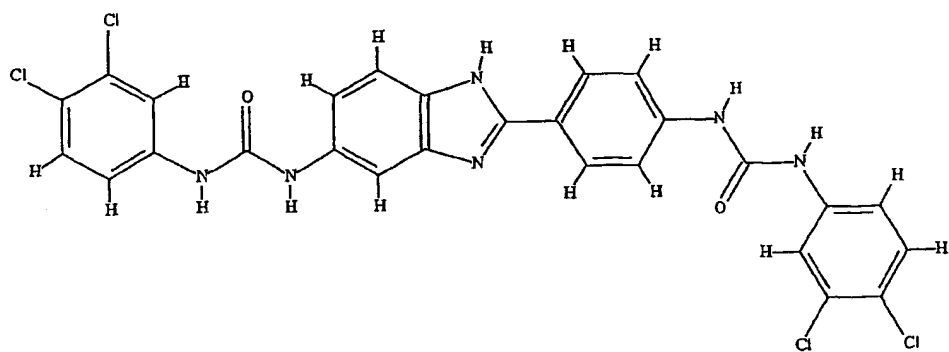
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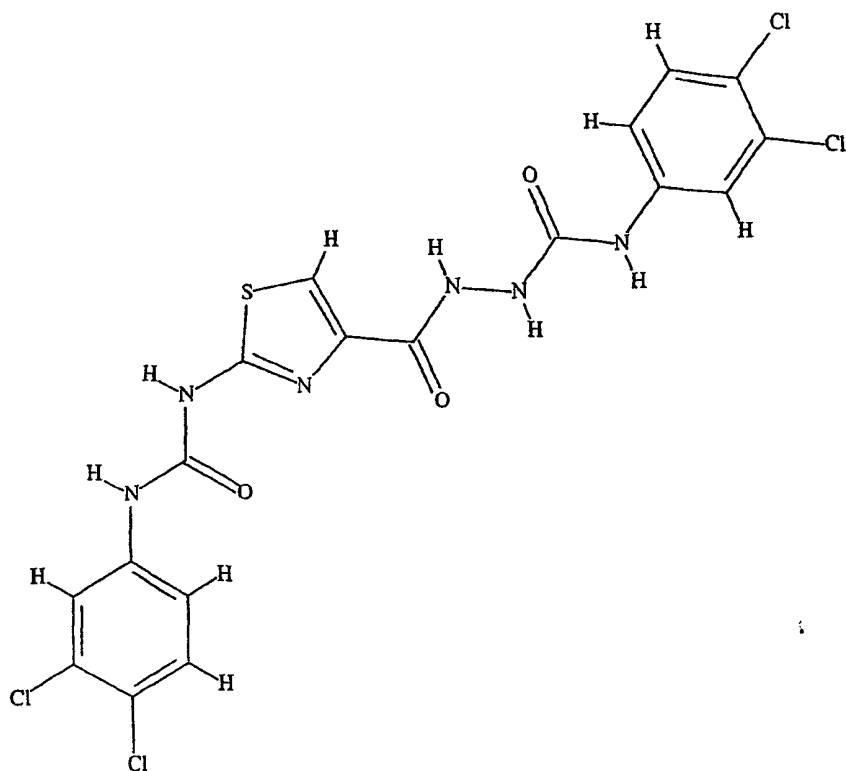
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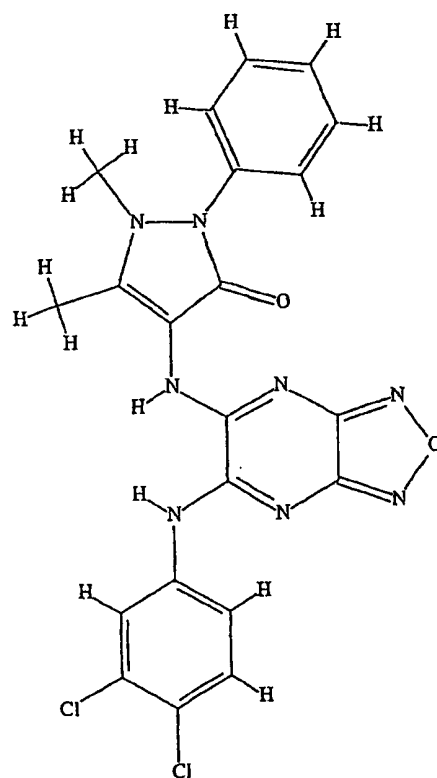
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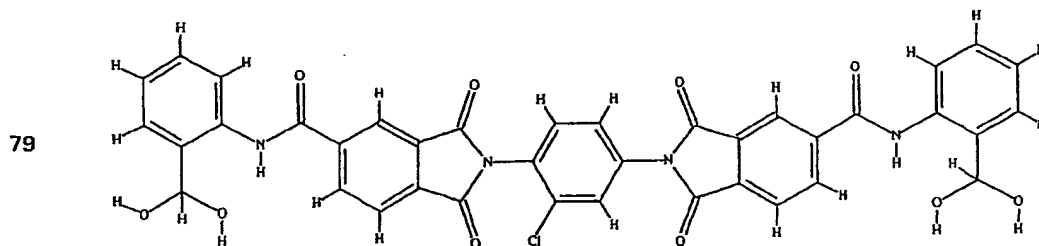
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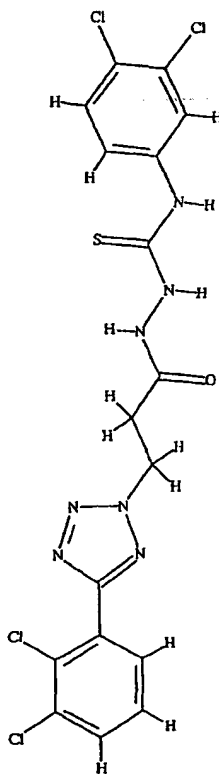
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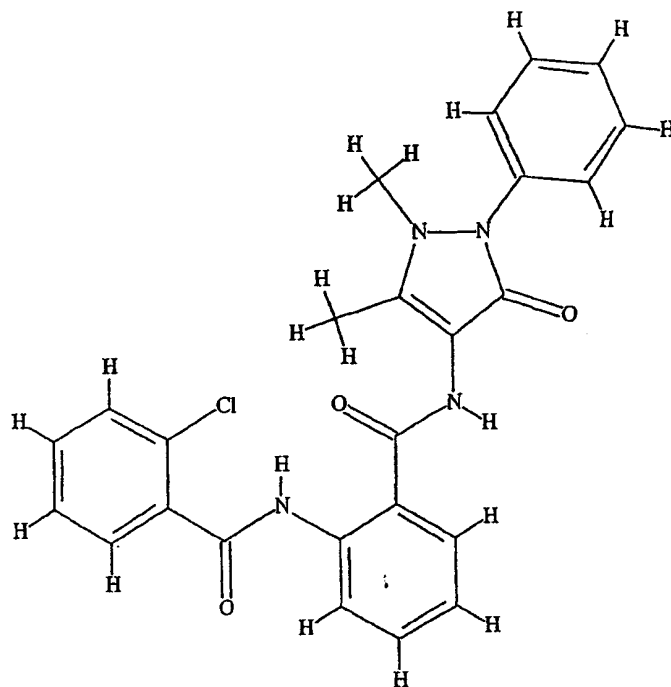


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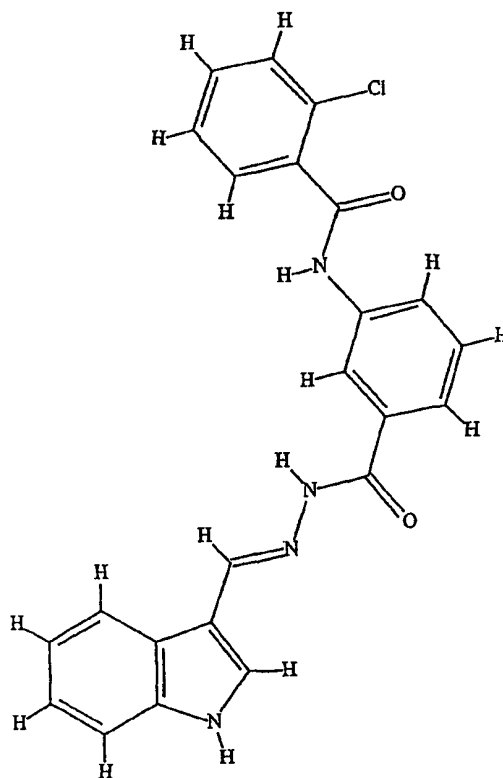


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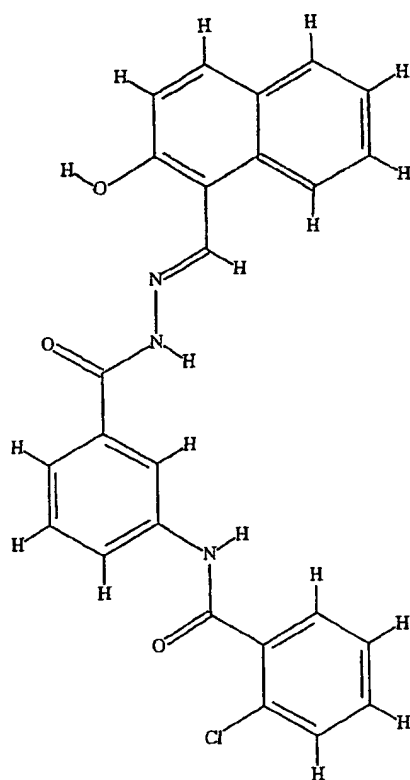
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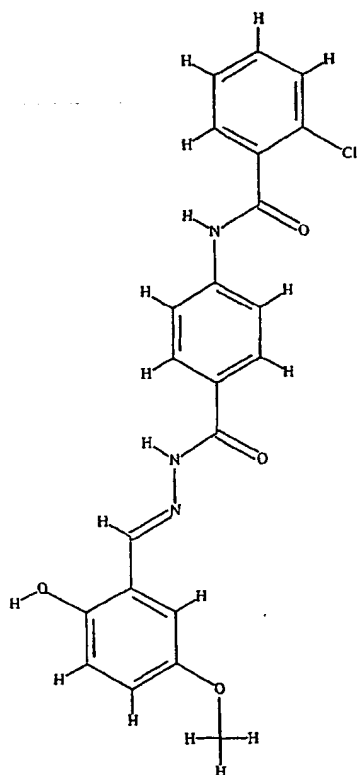
**SUBSTITUTE SHEET (RULE 26)**

42/248

83

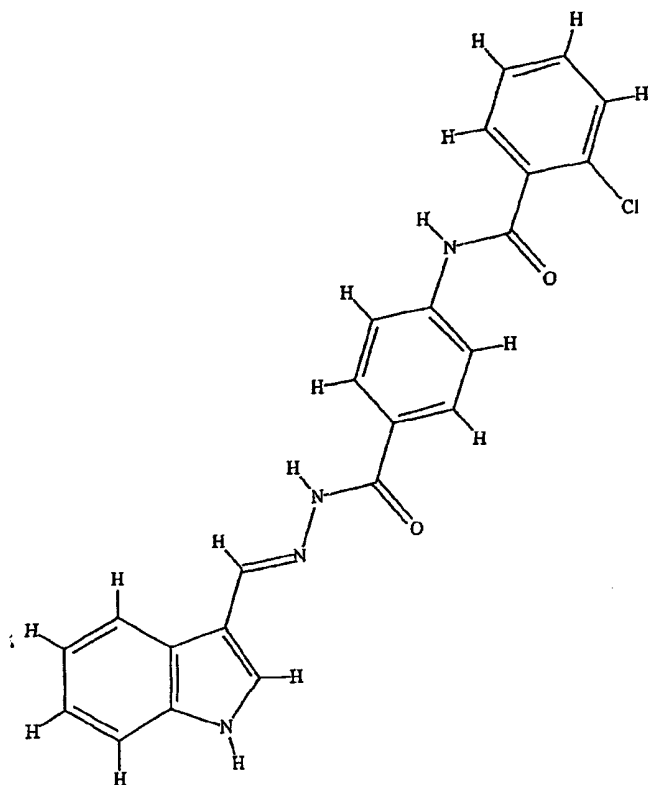


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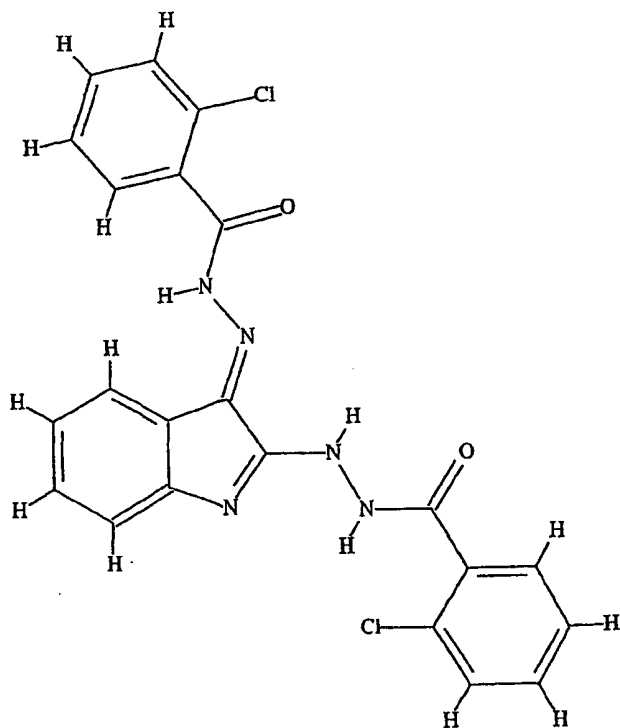


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85

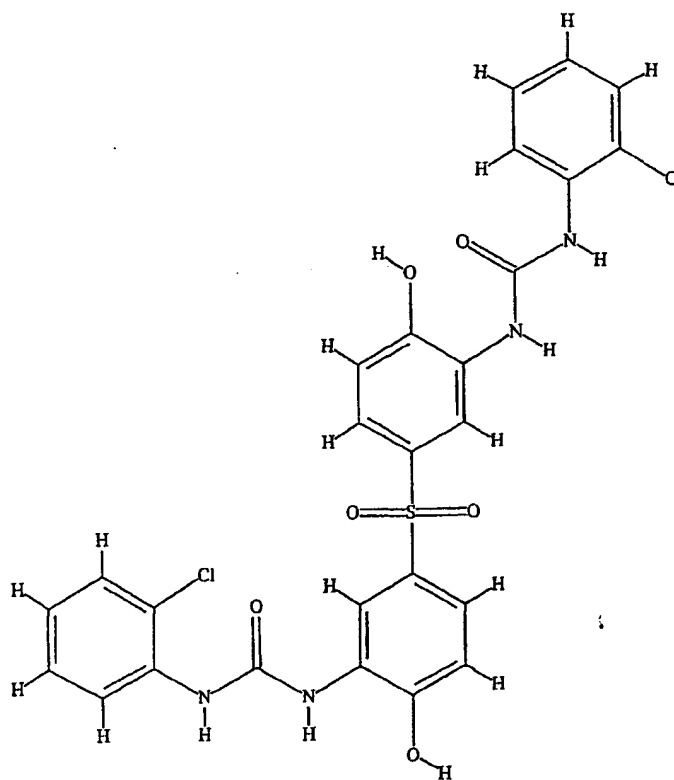


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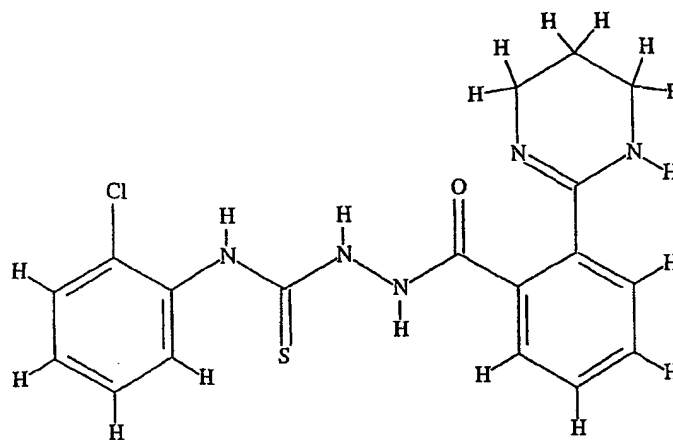


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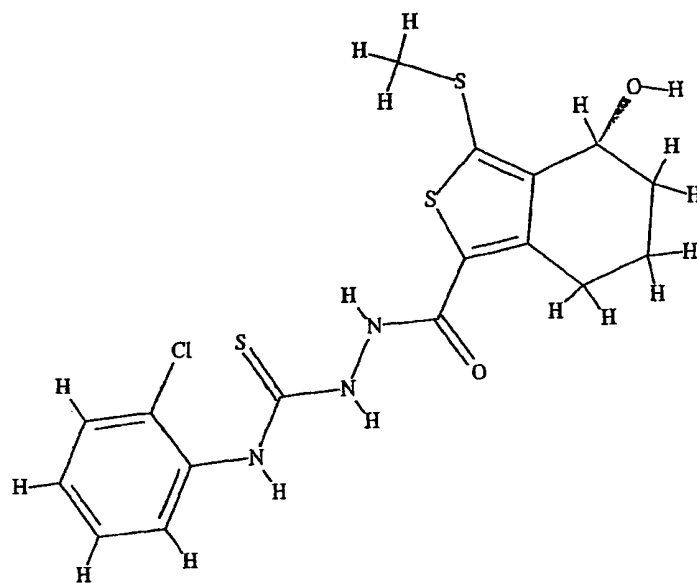
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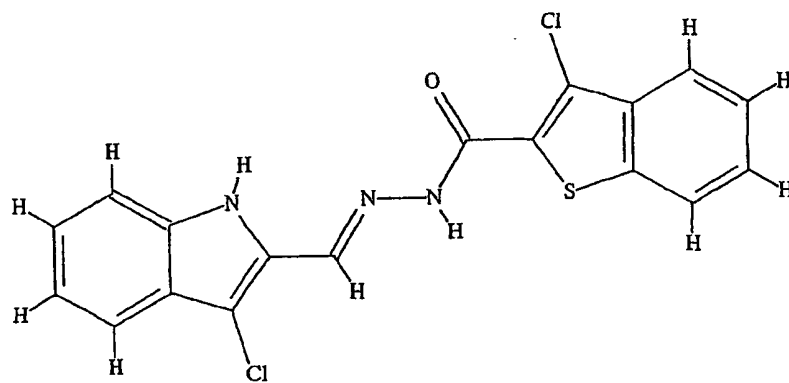
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89



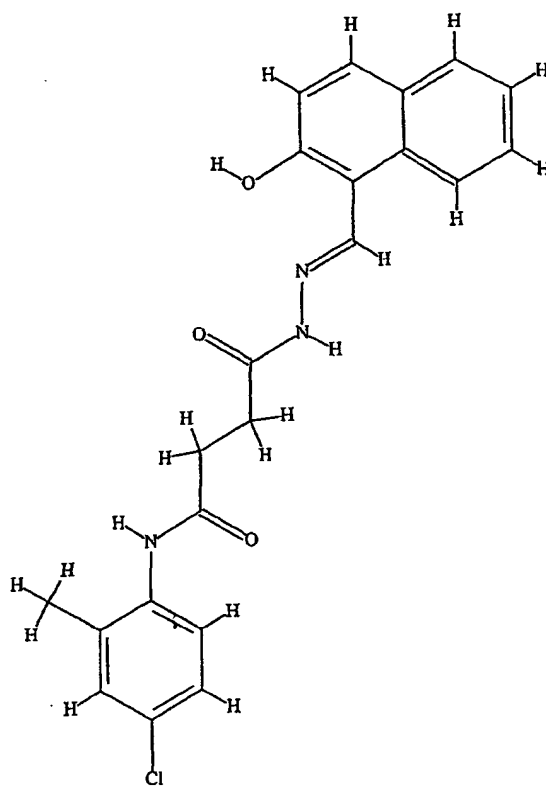
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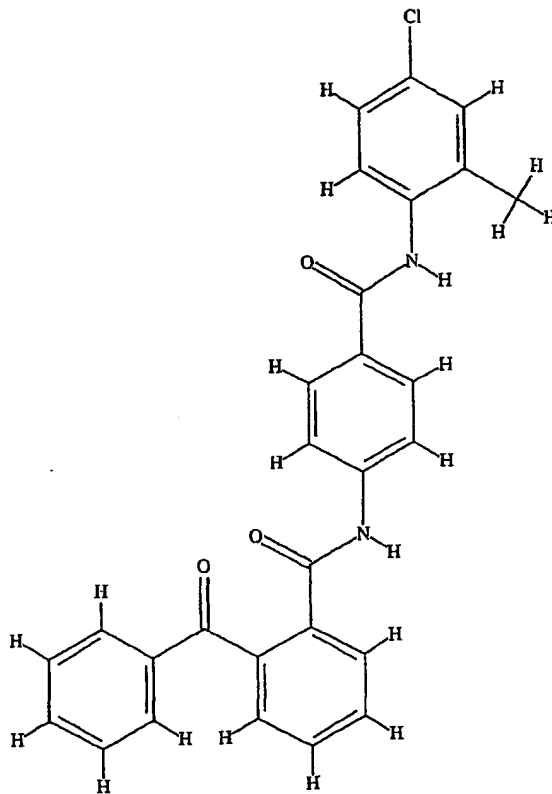


46/248

91

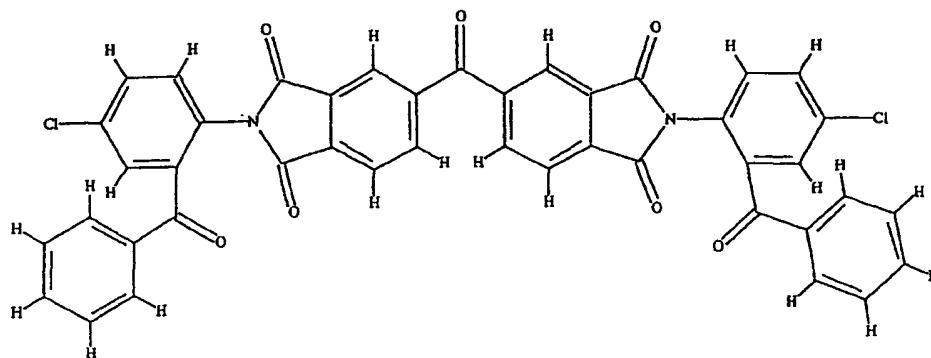


92

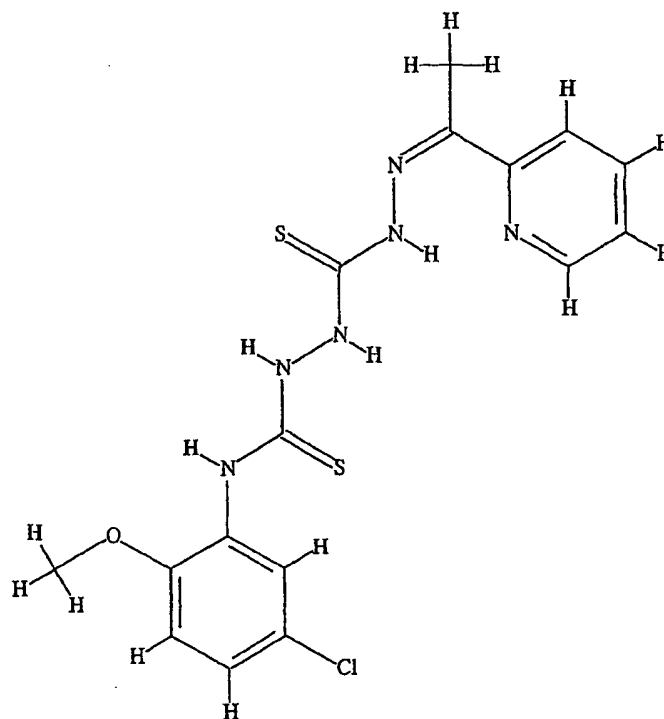


SUBSTITUTE SHEET (RULE 26)

93



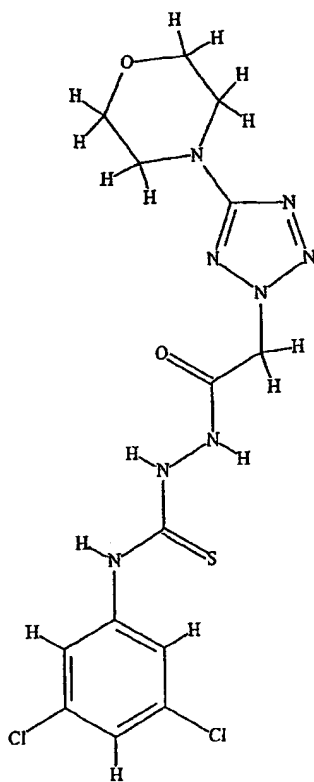
94



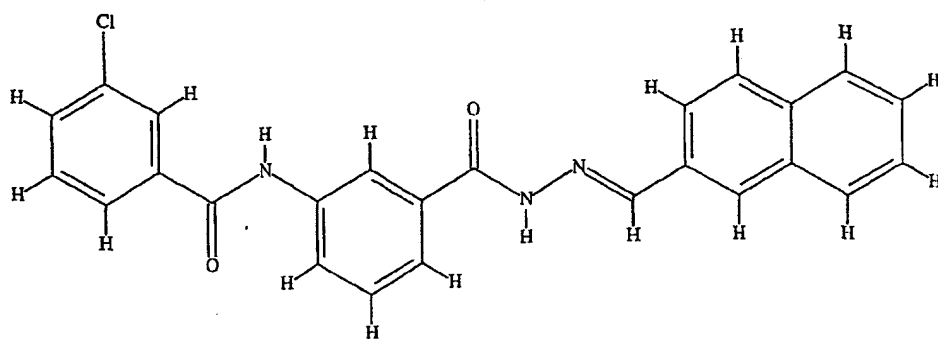
SUBSTITUTE SHEET (RULE 26)

48/248

95

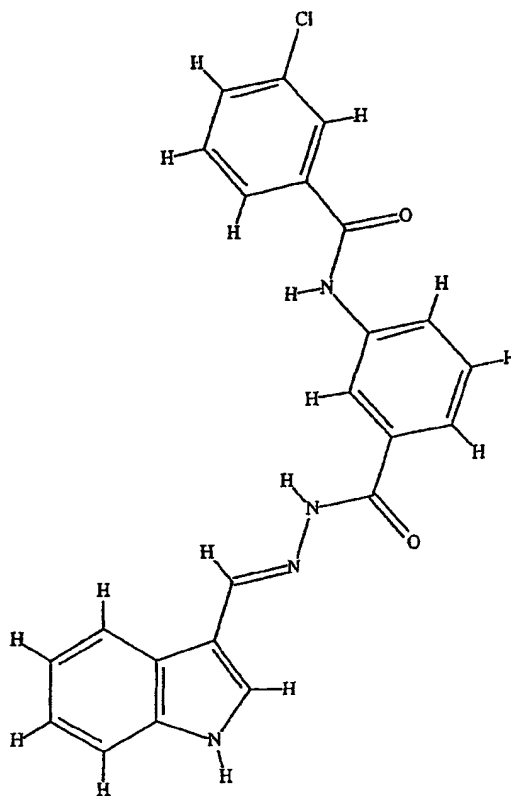


96

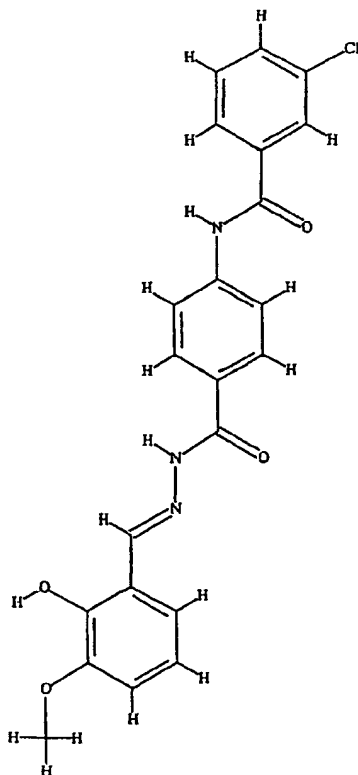


SUBSTITUTE SHEET (RULE 26)

97

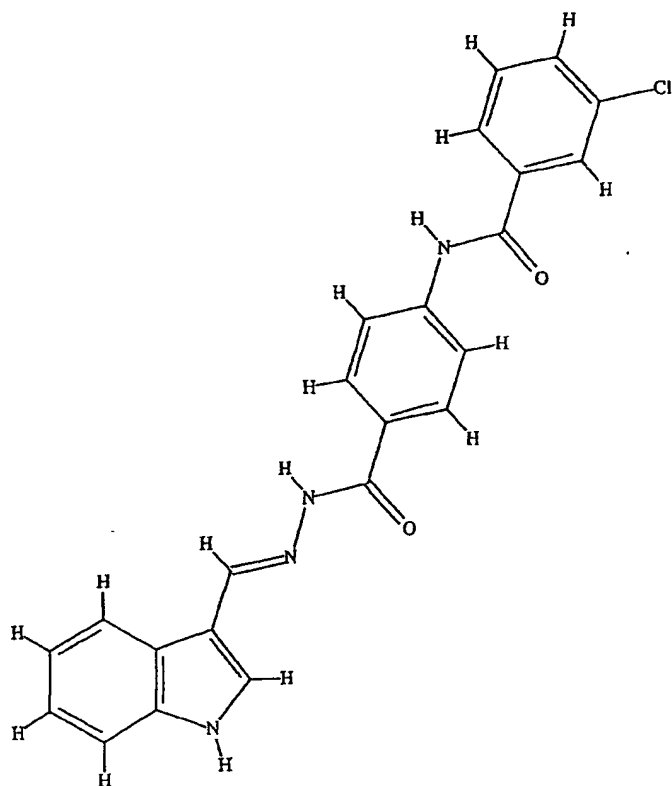


98

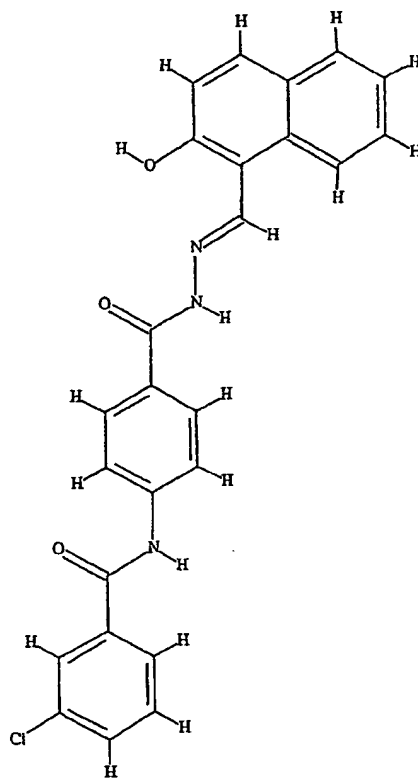


SUBSTITUTE SHEET (RULE 26)

99

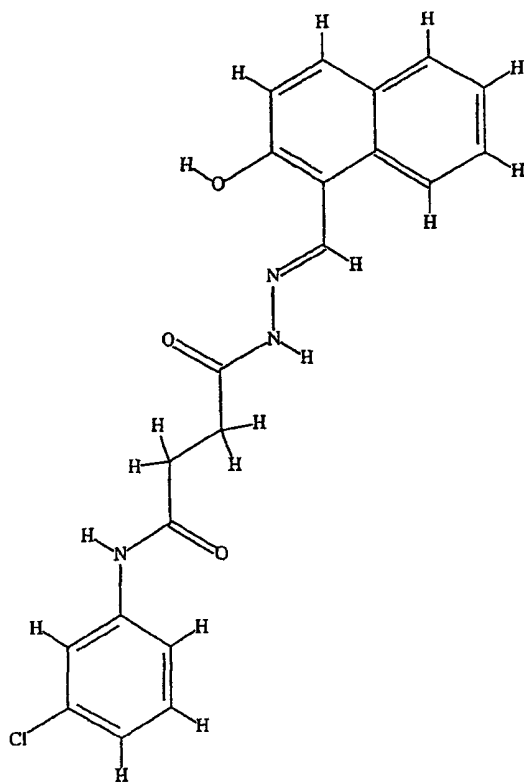


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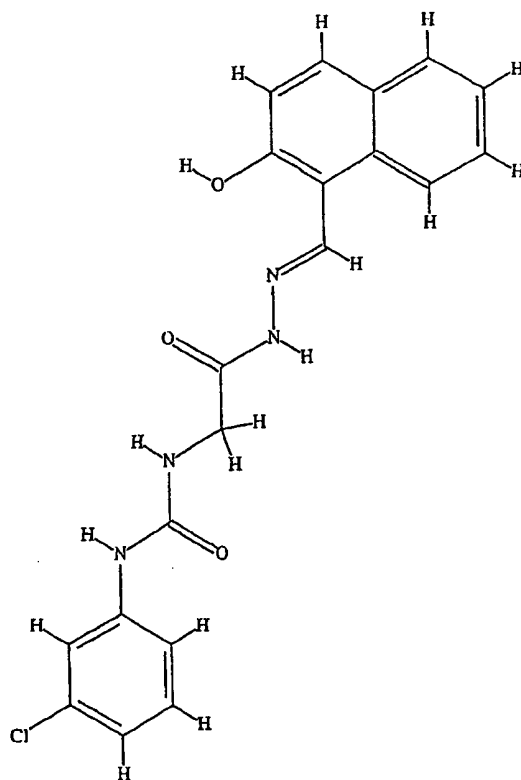


SUBSTITUTE SHEET (RULE 26)

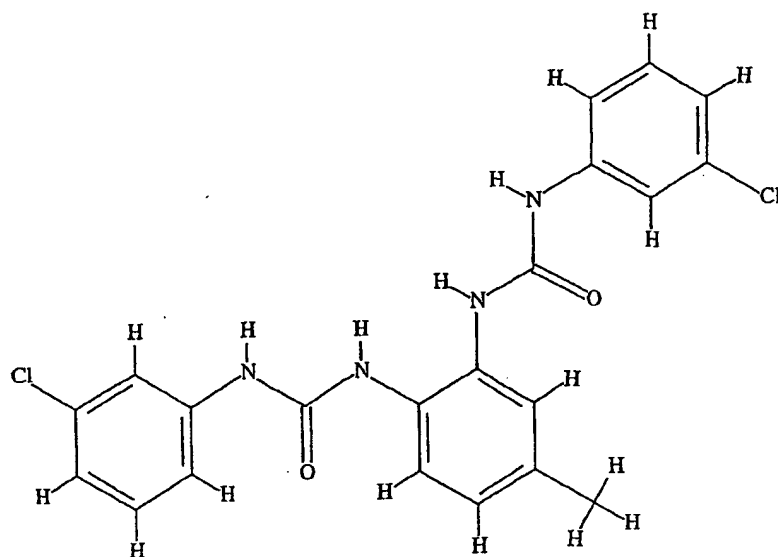
101



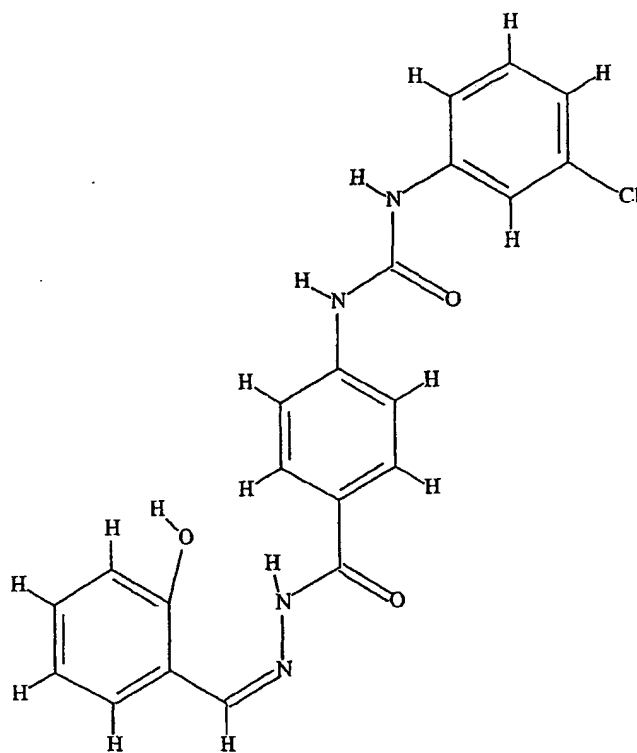
102



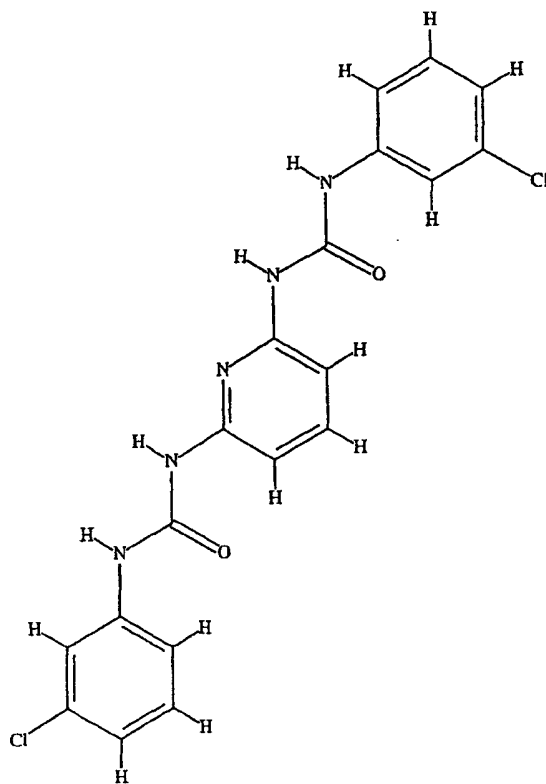
103



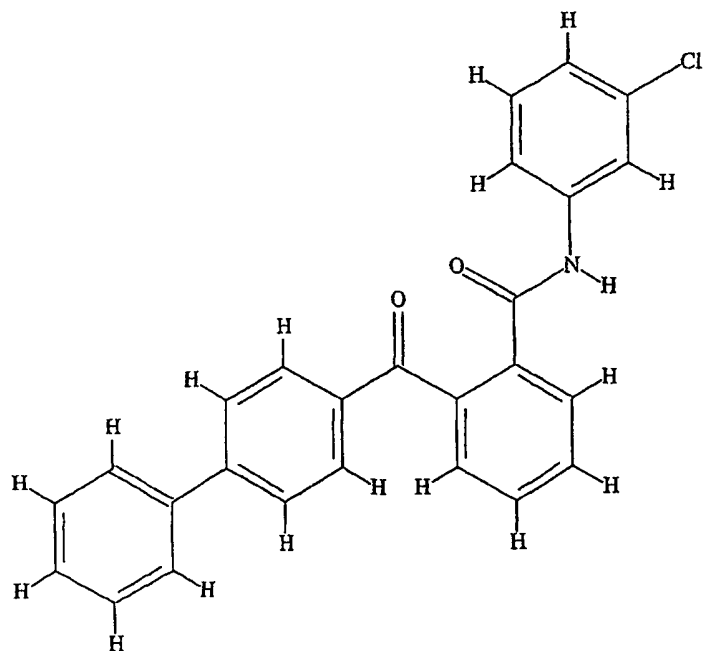
104



105



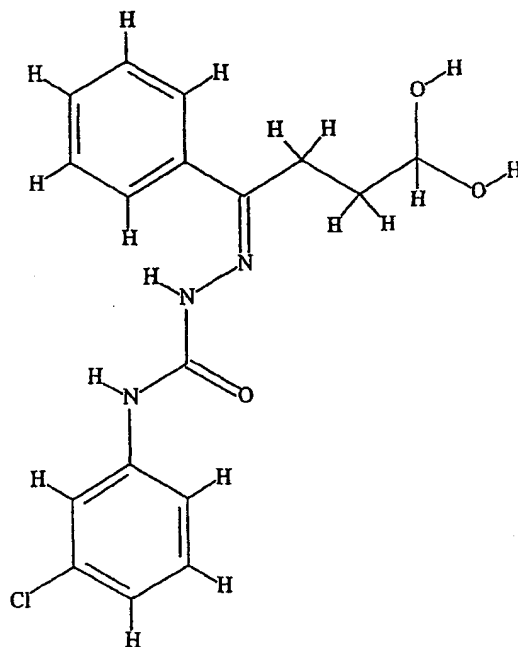
106



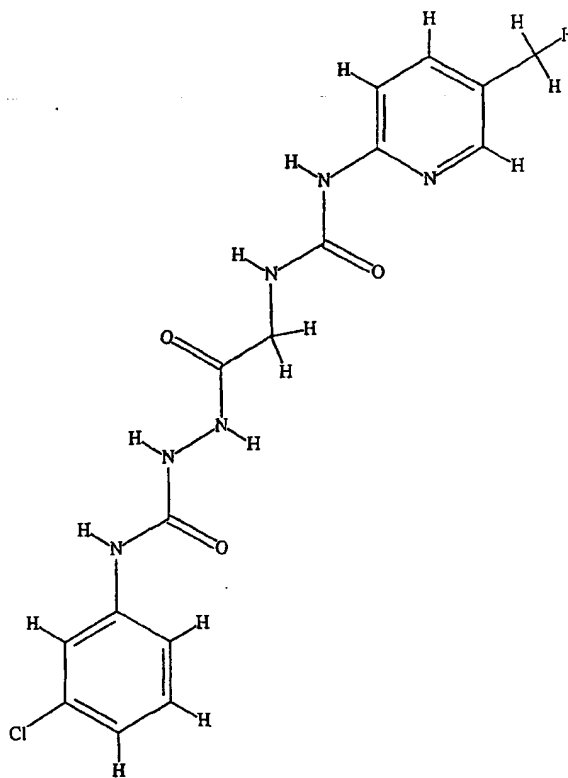


54/248

107

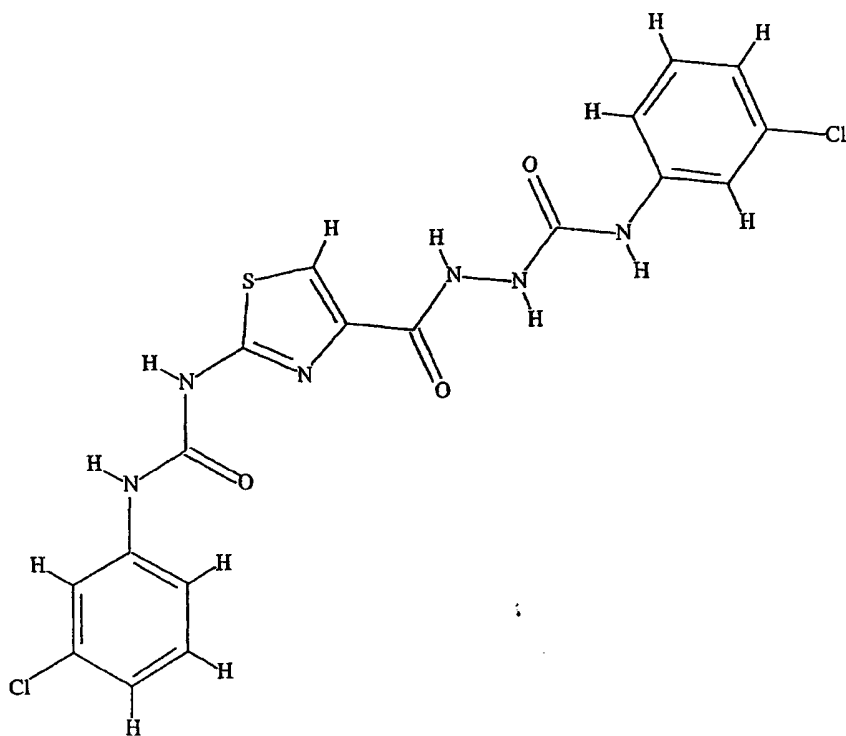


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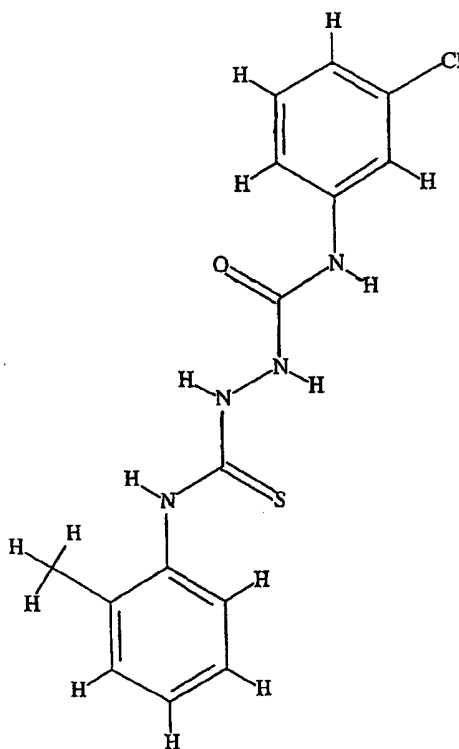


SUBSTITUTE SHEET (RULE 26)

109



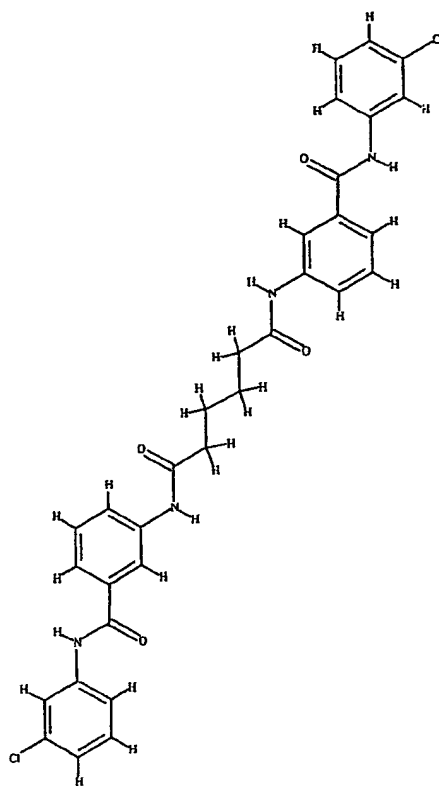
110



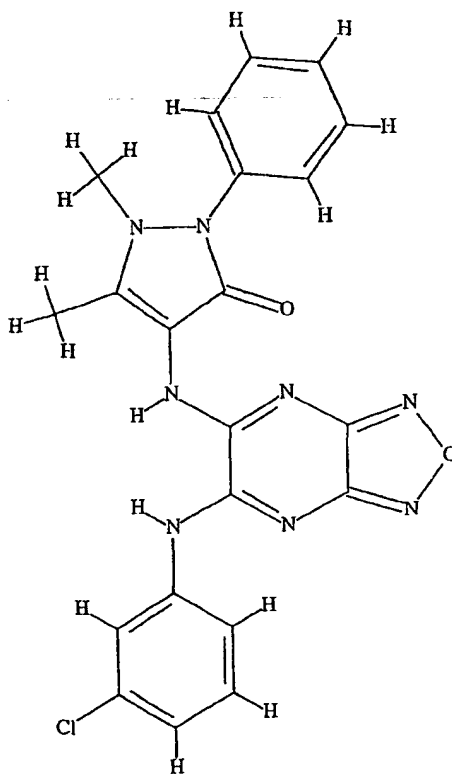
SUBSTITUTE SHEET (RULE 26)

56/248

111



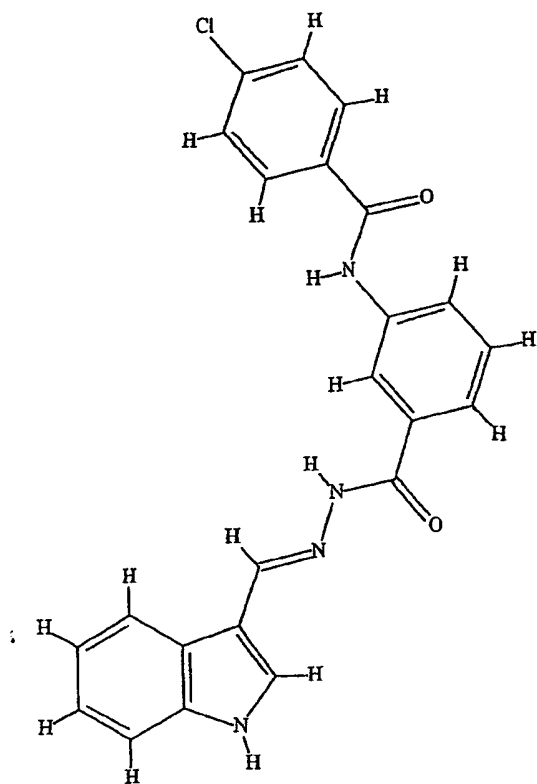
112



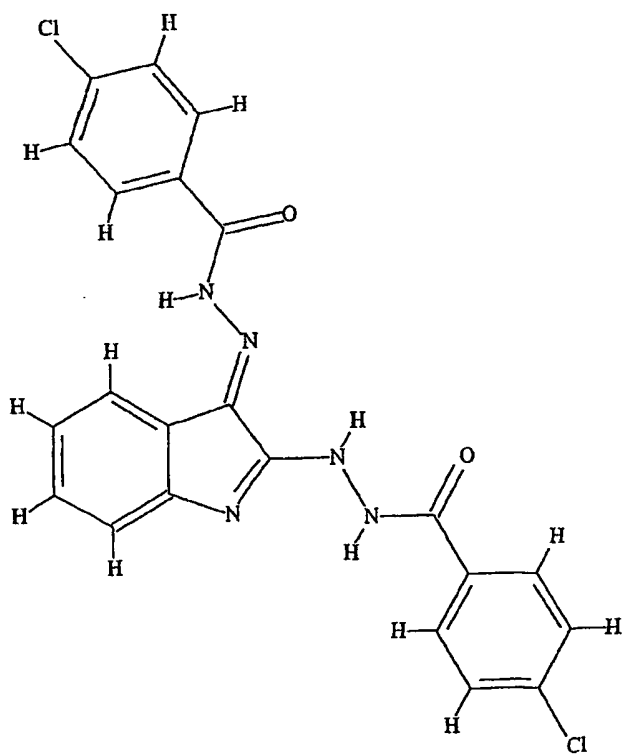
SUBSTITUTE SHEET (RULE 26)

57/248

113

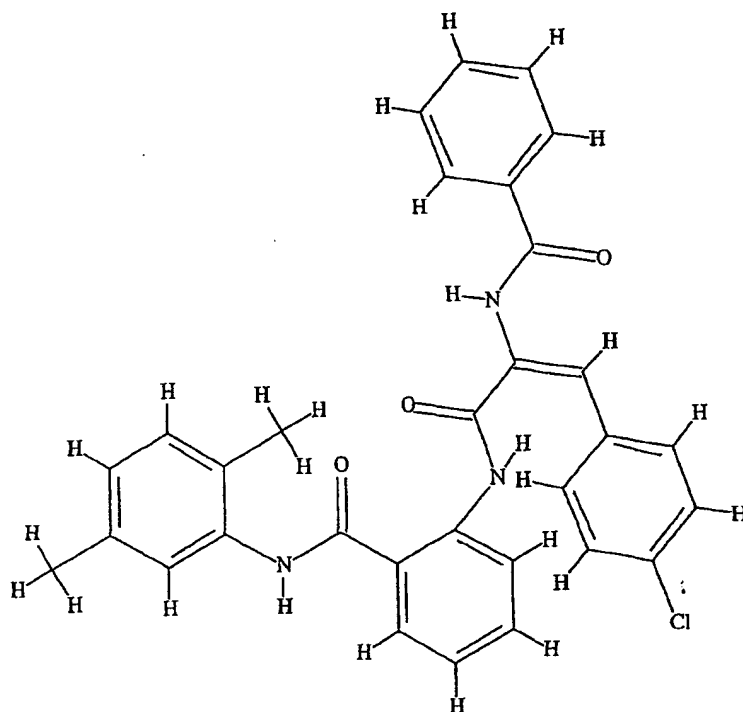


114

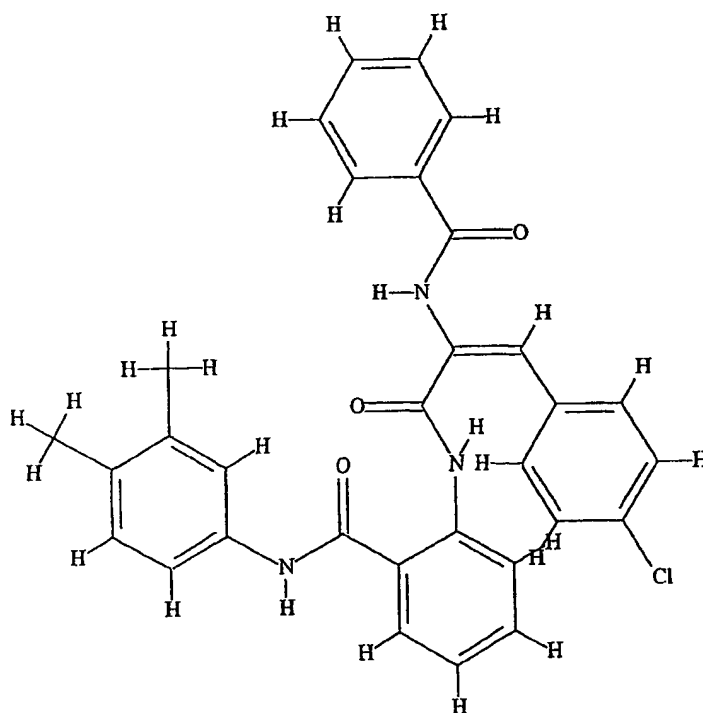


SUBSTITUTE SHEET (RULE 26)

115

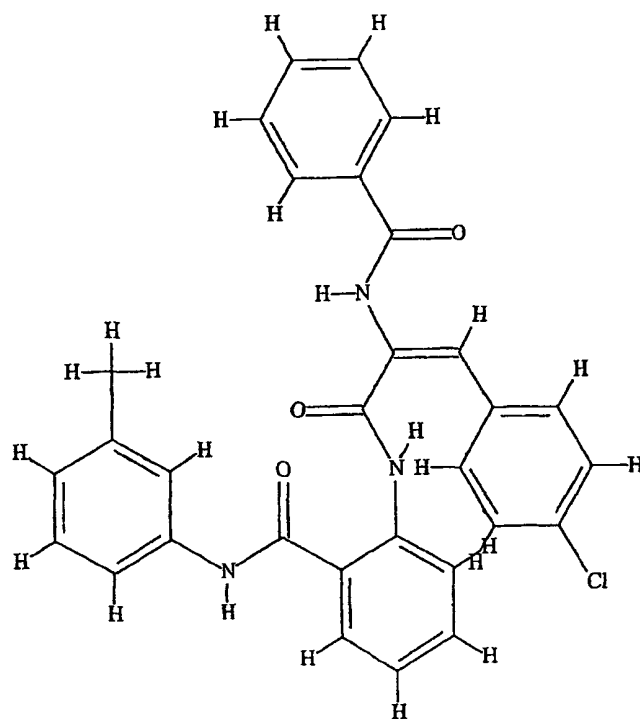


116

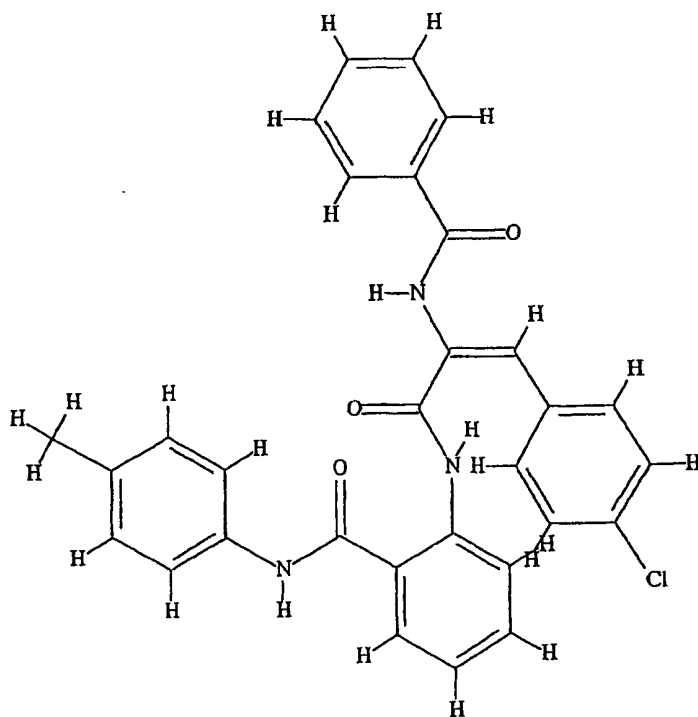


59/248

117

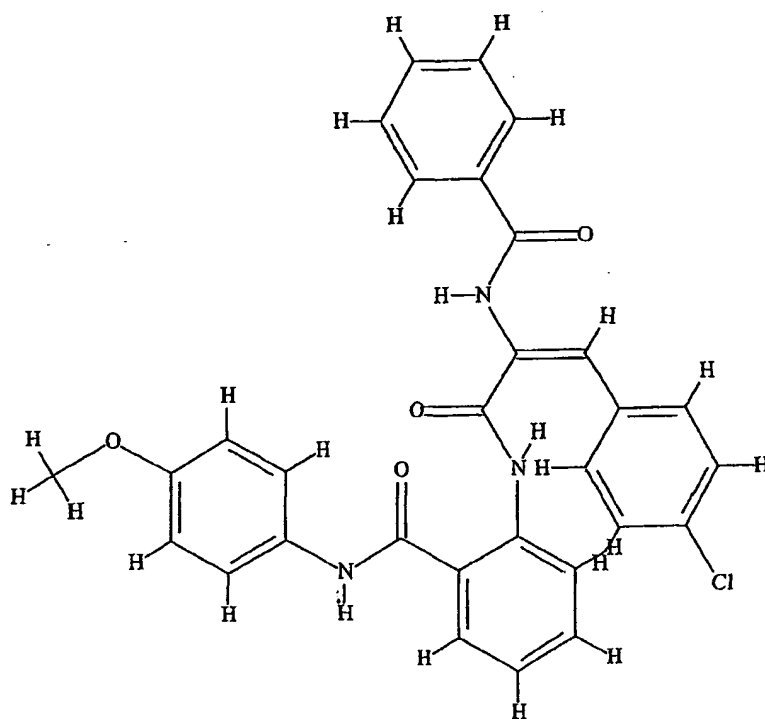


118

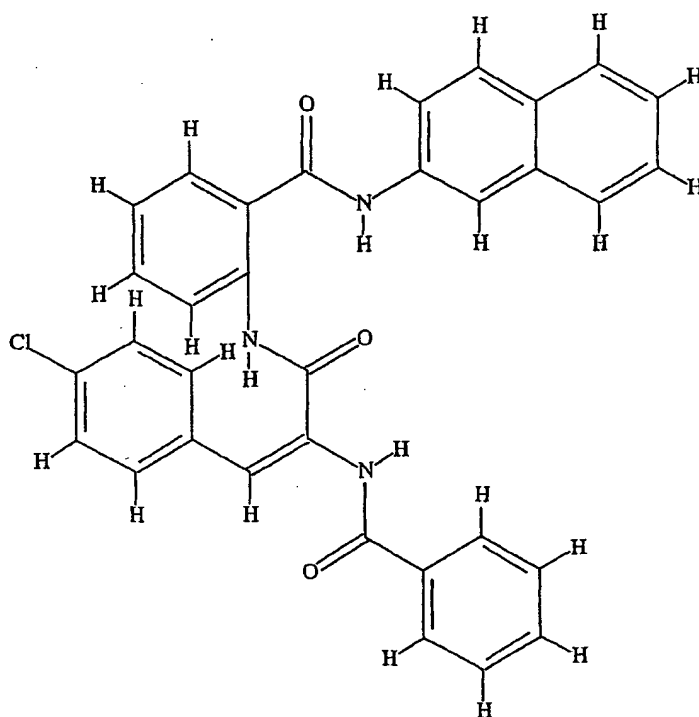


SUBSTITUTE SHEET (RULE 26)

119

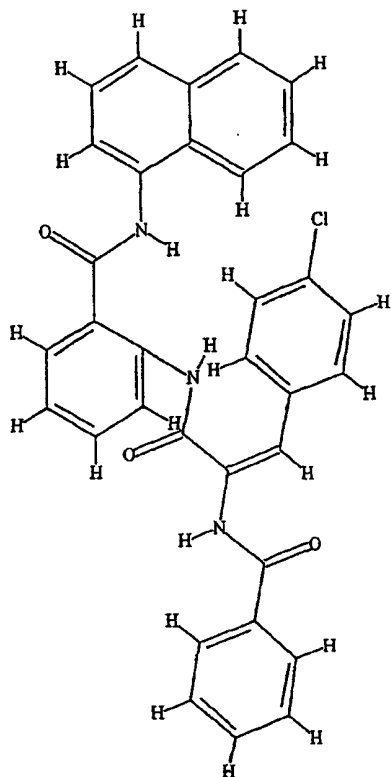


120

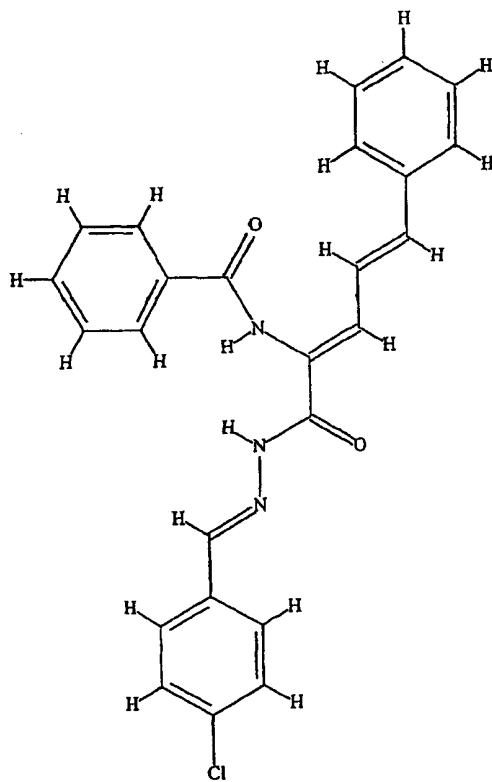


61/248

121



122

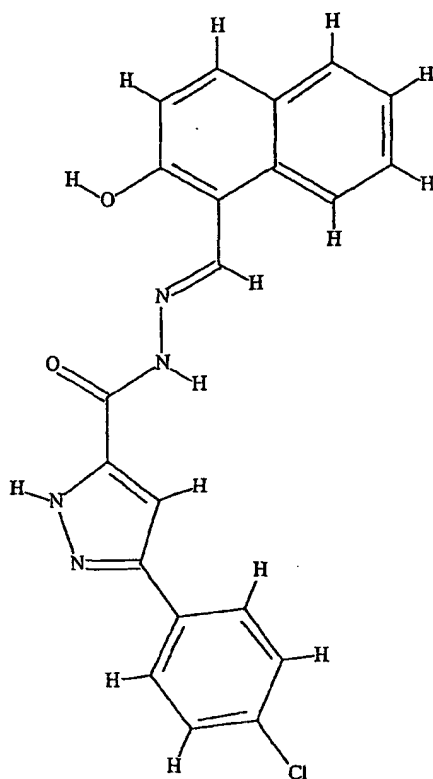


SUBSTITUTE SHEET (RULE 26)

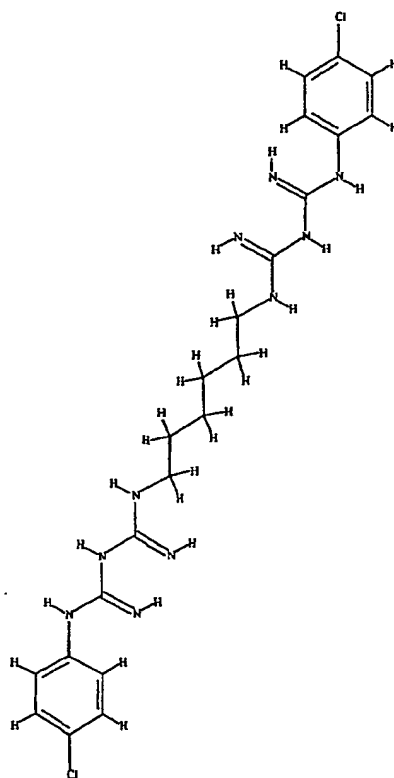


62/248

123

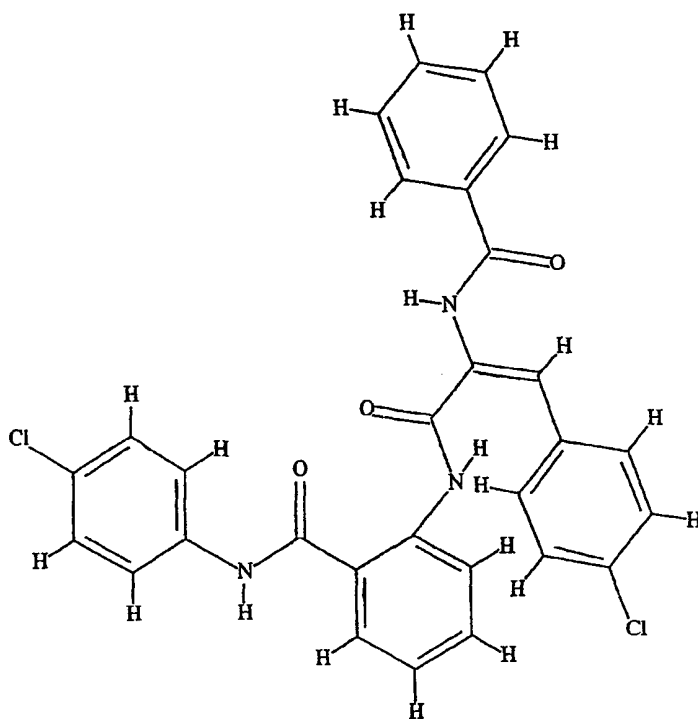


124

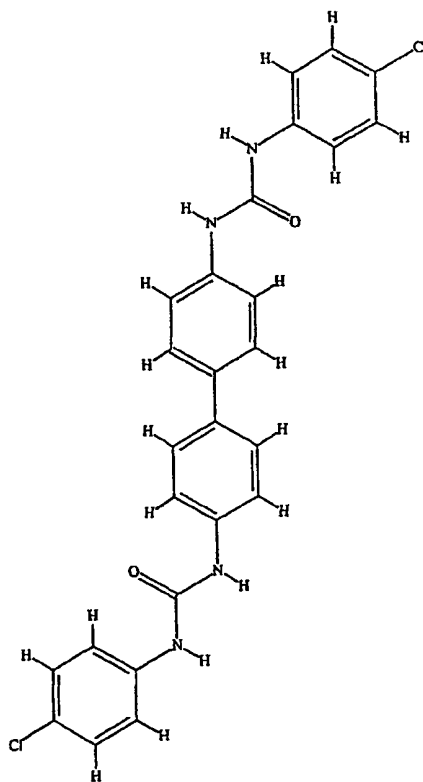


SUBSTITUTE SHEET (RULE 26)

125



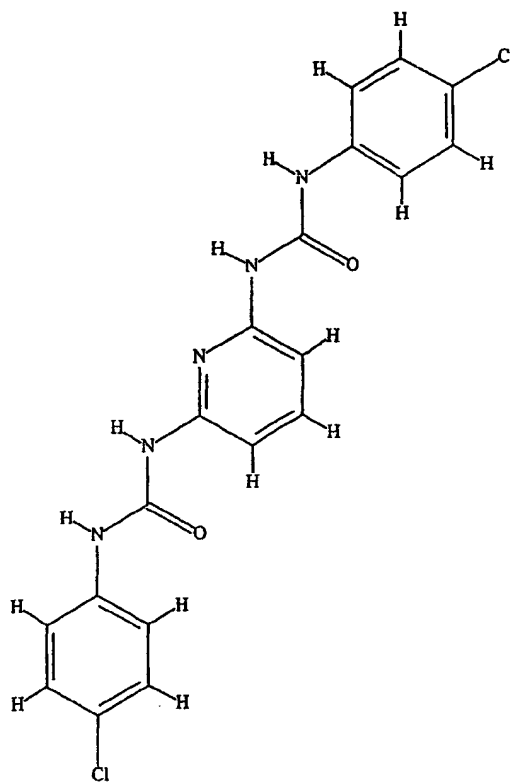
126



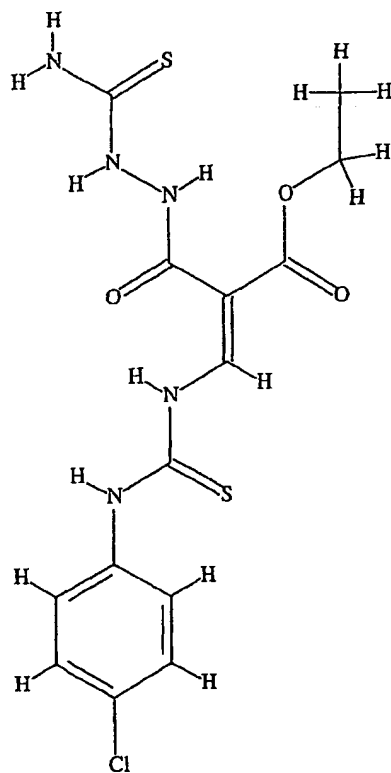
SUBSTITUTE SHEET (RULE 26)

64/248

127



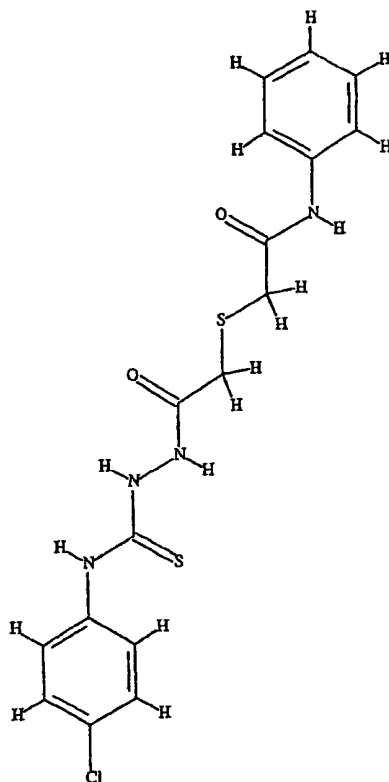
128



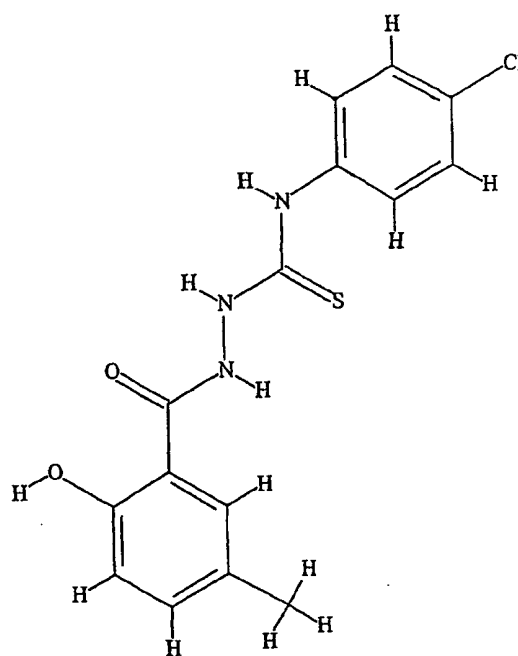
SUBSTITUTE SHEET (RULE 26)

65/248

129



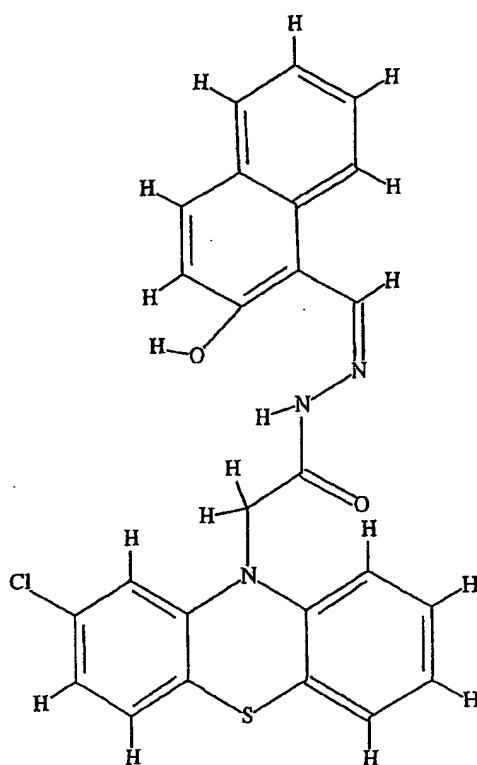
130



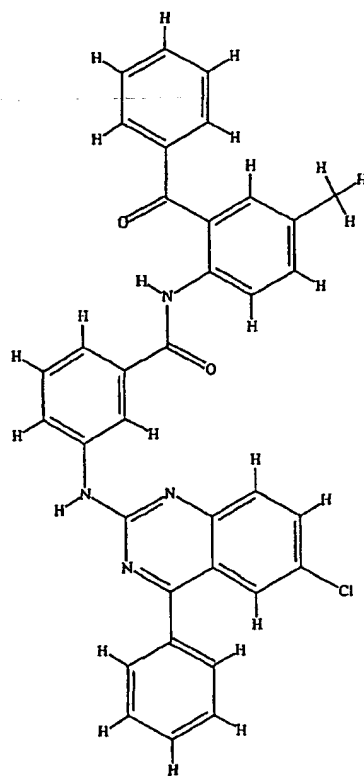
SUBSTITUTE SHEET (RULE 26)

66/248

131

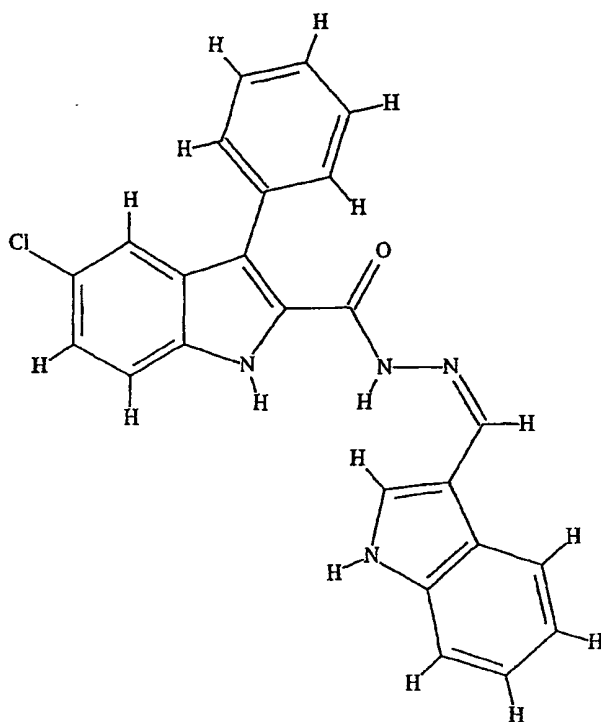


132

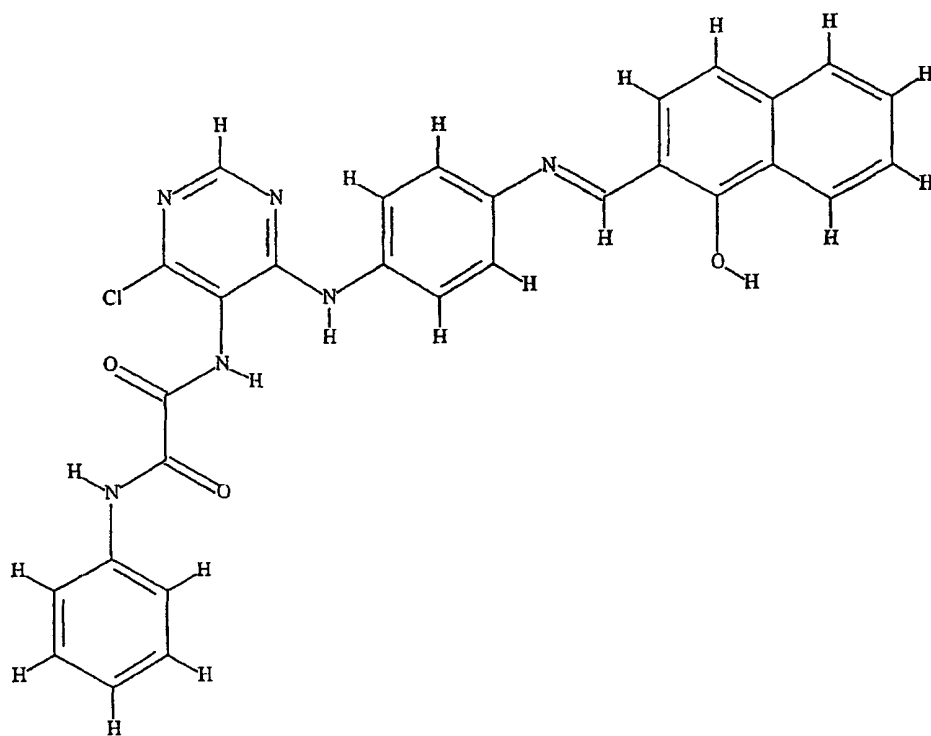


SUBSTITUTE SHEET (RULE 26)

133

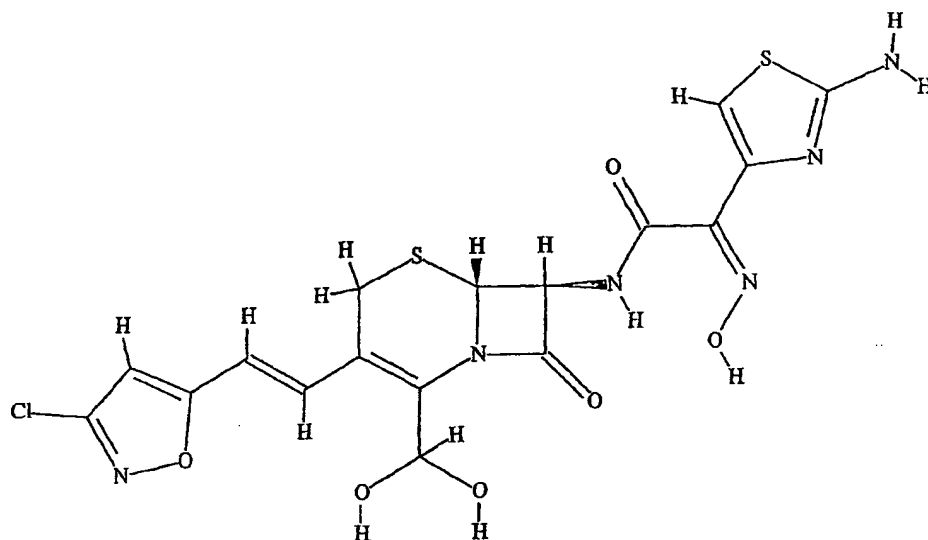


134

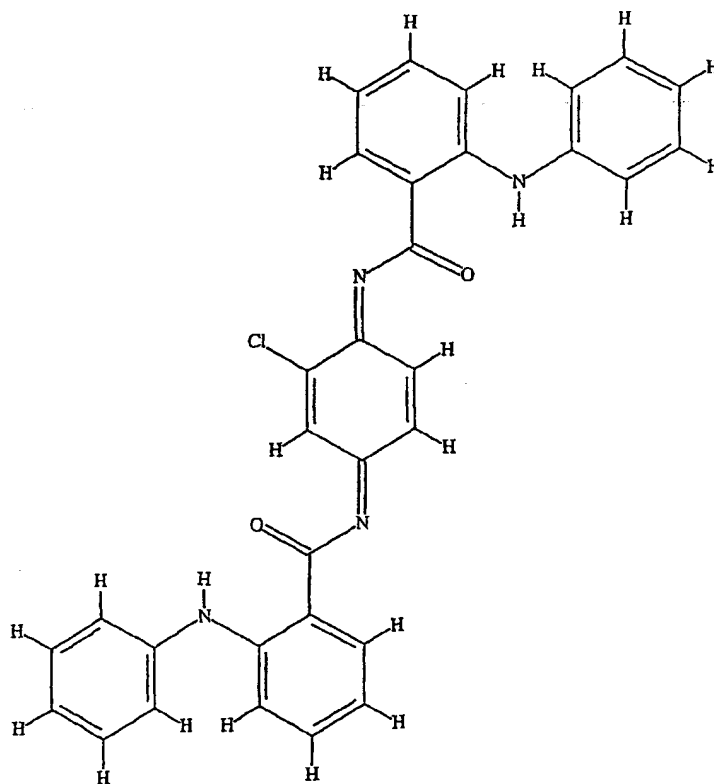


SUBSTITUTE SHEET (RULE 26)

135

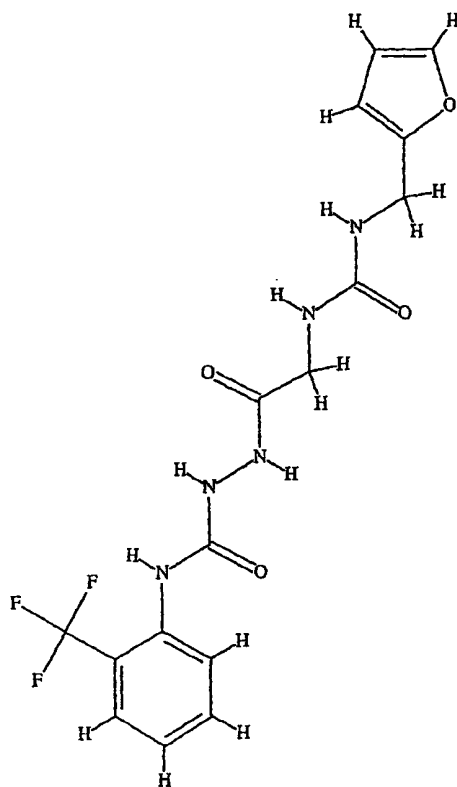


136

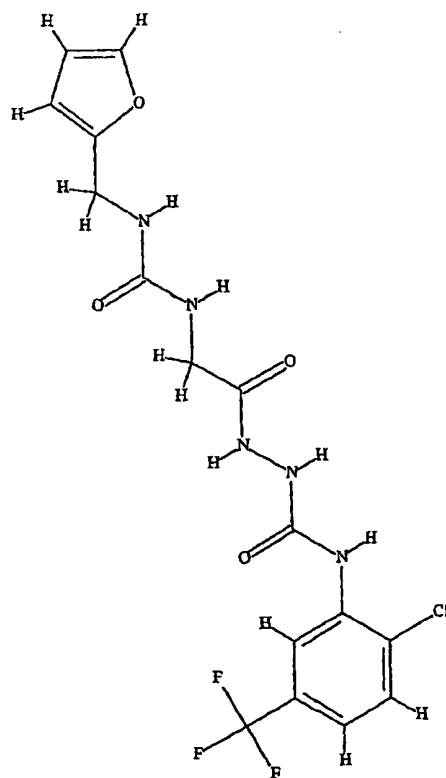


SUBSTITUTE SHEET (RULE 26)

137



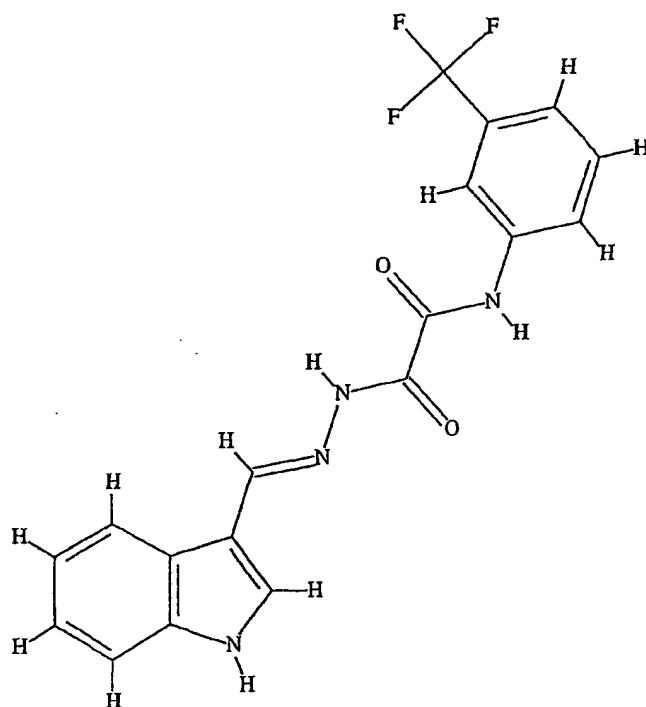
138



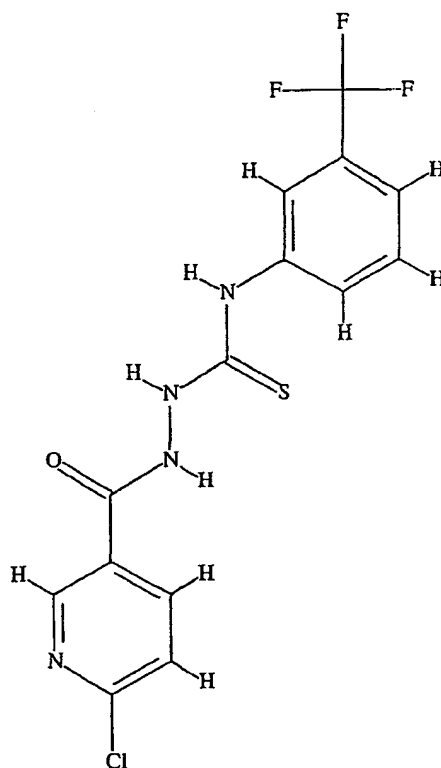
SUBSTITUTE SHEET (RULE 26)



139

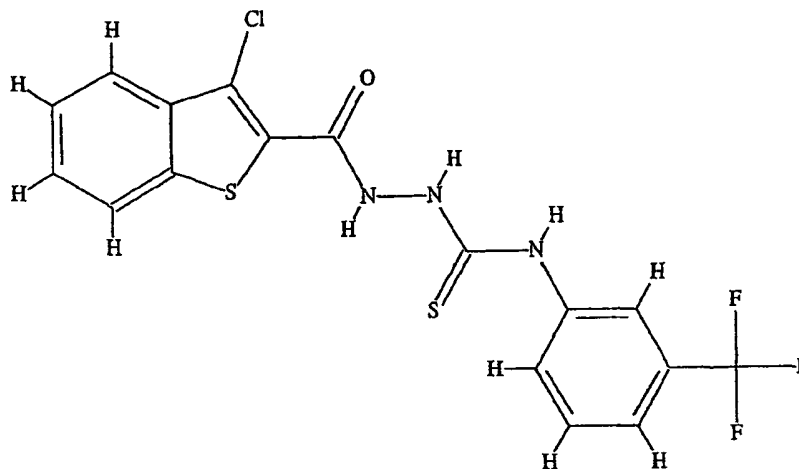


140

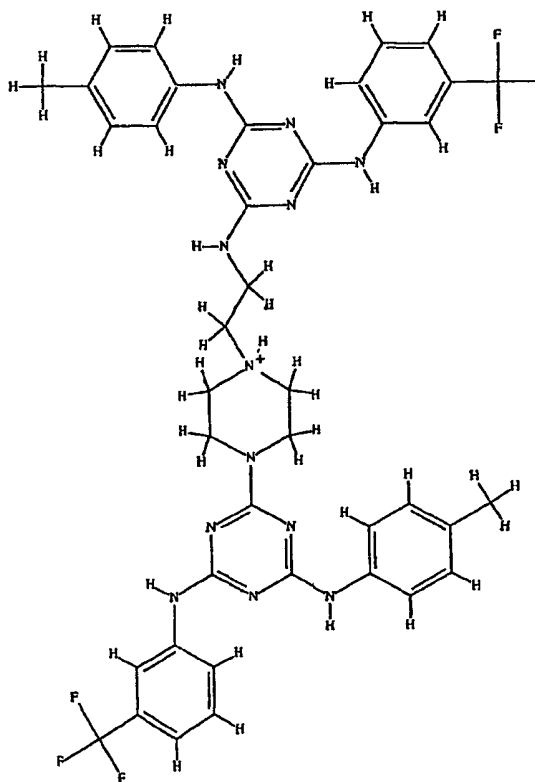


SUBSTITUTE SHEET (RULE 26)

141



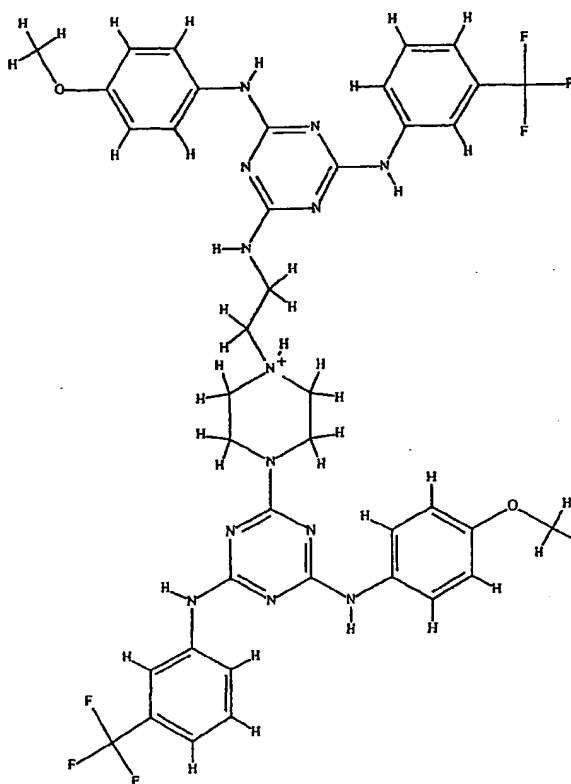
142



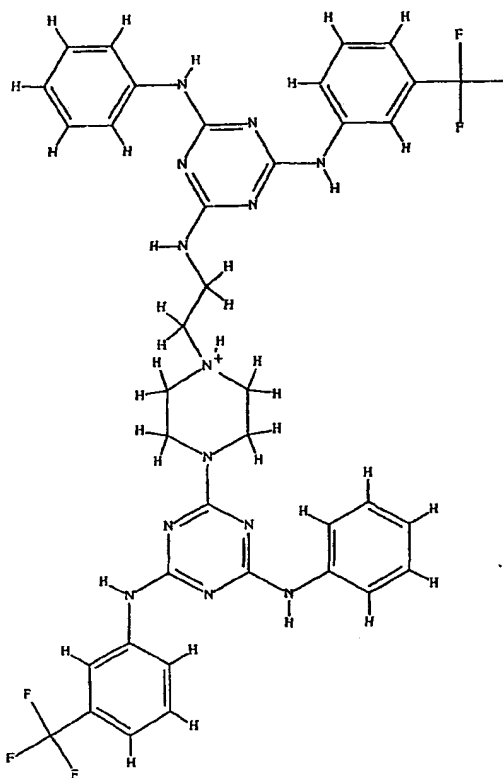
SUBSTITUTE SHEET (RULE 26)

72/248

143

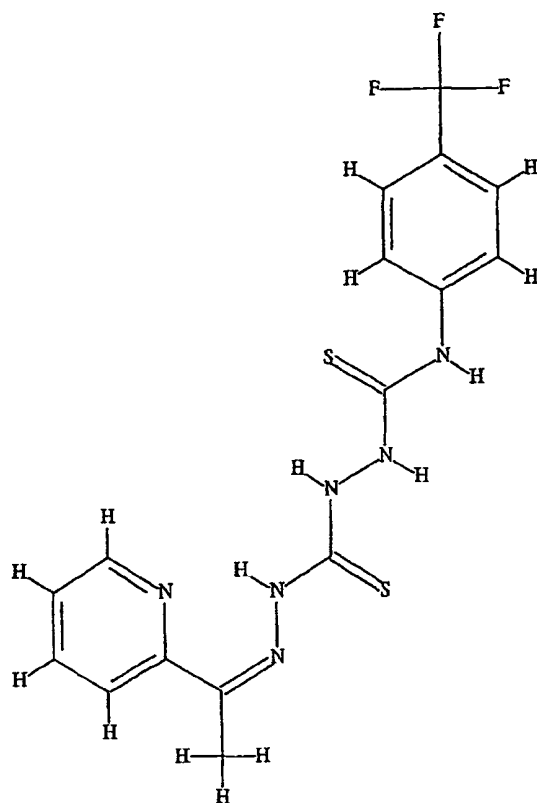


144

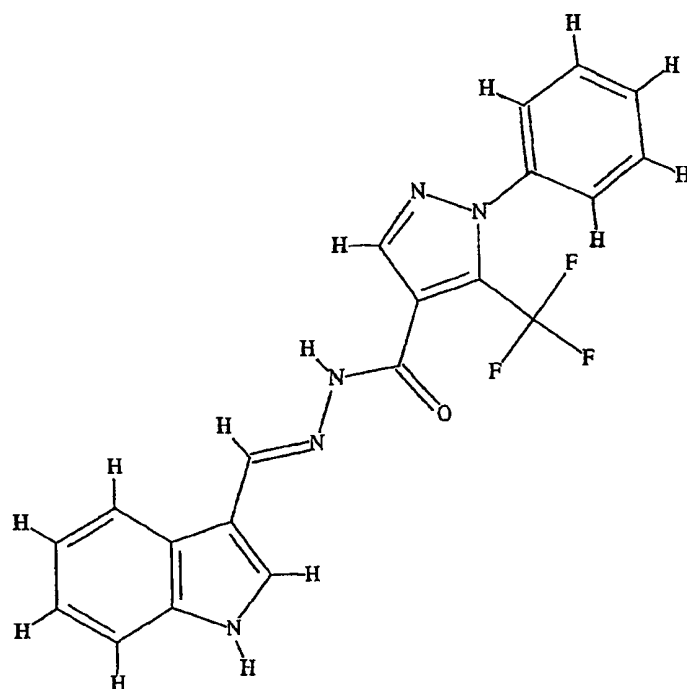


SUBSTITUTE SHEET (RULE 26)

145



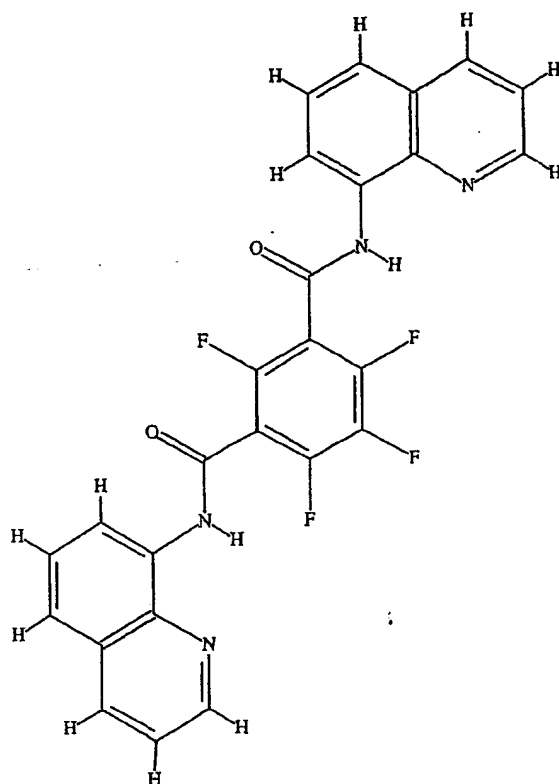
146



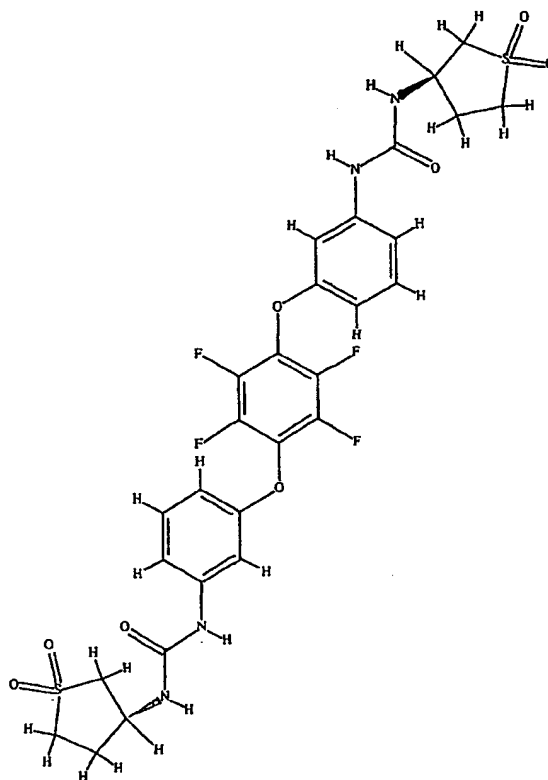
SUBSTITUTE SHEET (RULE 26)

74/248

147

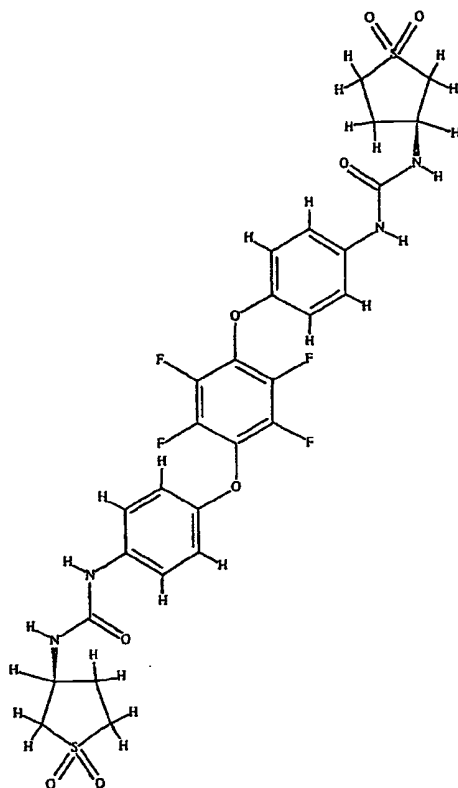


148

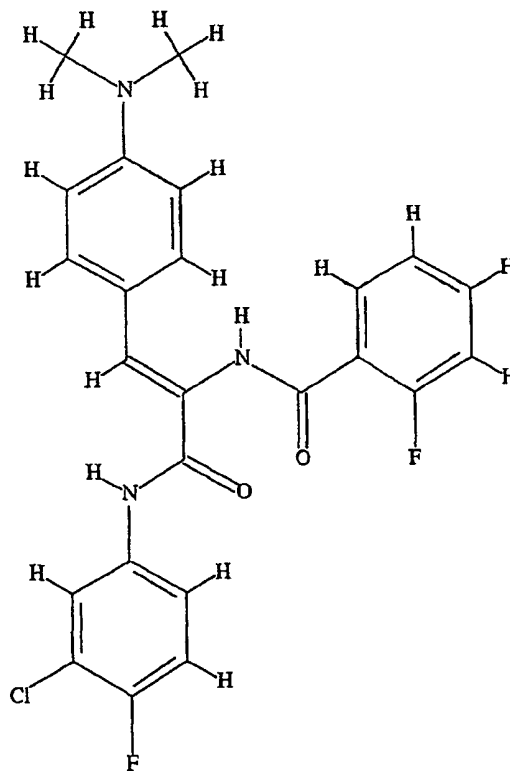


SUBSTITUTE SHEET (RULE 26)

149



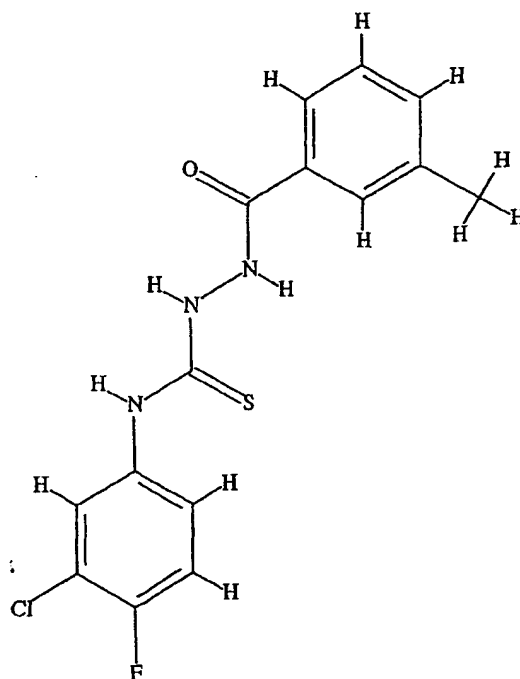
150



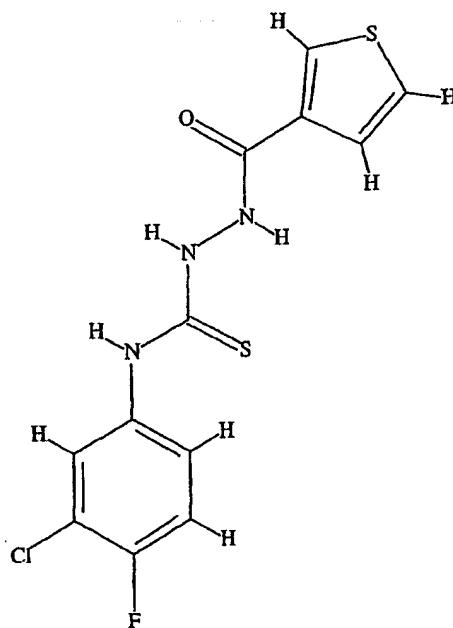
SUBSTITUTE SHEET (RULE 26)

76/248

151



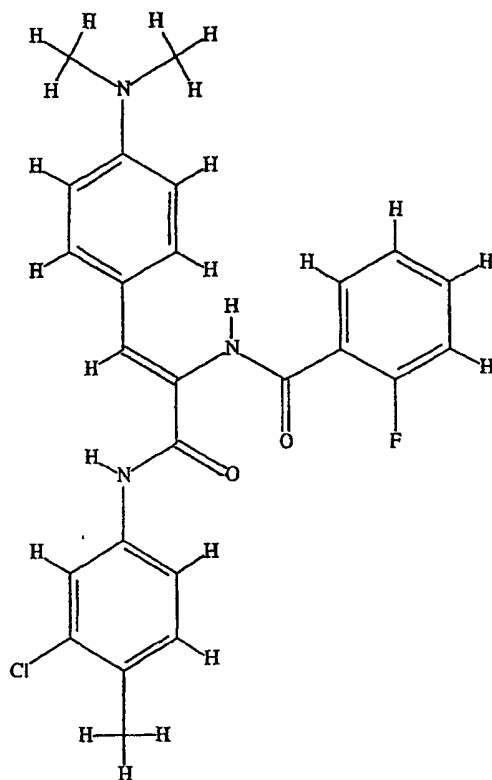
152



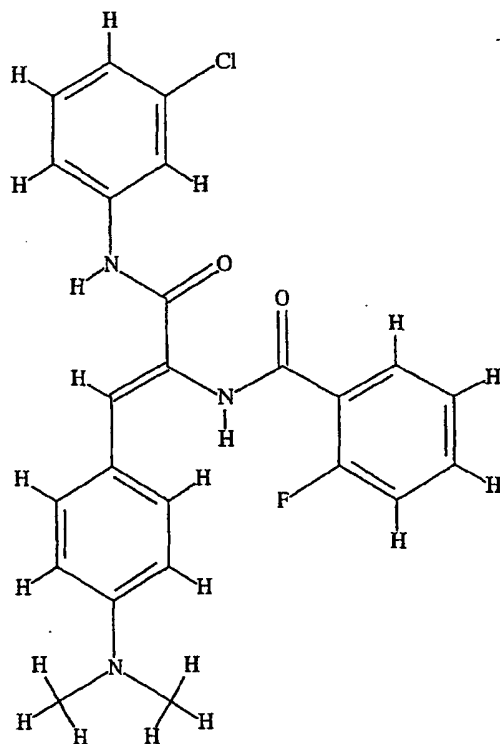
SUBSTITUTE SHEET (RULE 26)

77/248

153



154



SUBSTITUTE SHEET (RULE 26)

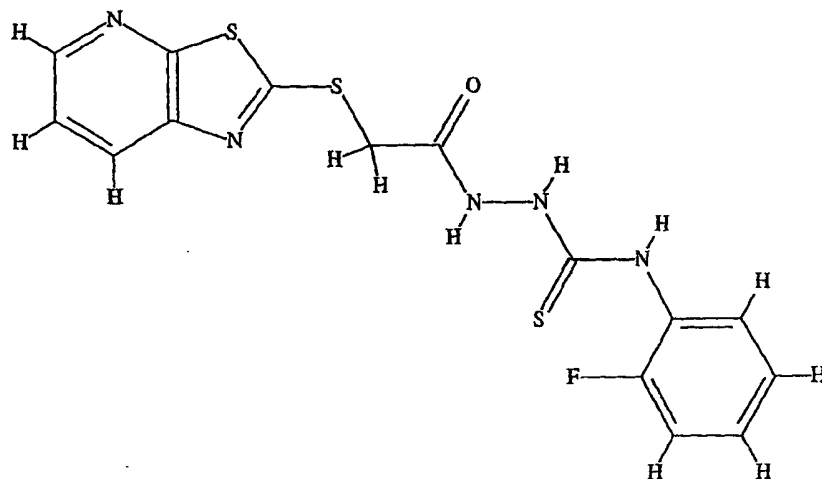


FC1=CC=C(C=C1)NC(=O)C2=CC=CC=C2NC(=O)C3=CC=C(C=C3)N(C)C

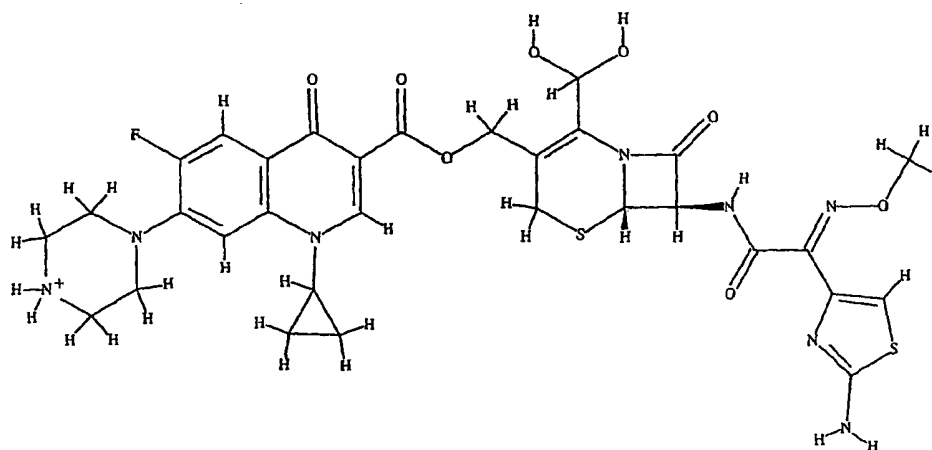
The chemical structure shows a benzene ring substituted with three fluorine atoms at the 2, 4, and 6 positions. This ring is connected at the 1-position to a methylene group, which is in turn connected to a hydrazinecarboxamide group. The hydrazine group consists of two nitrogen atoms linked together, with one nitrogen attached to the methylene group and the other attached to a carbonyl group (C=O). The benzene ring has hydrogen atoms at positions 3 and 5.

BNSDOCID: <WO\_\_\_\_\_0168122A2\_I\_>

157

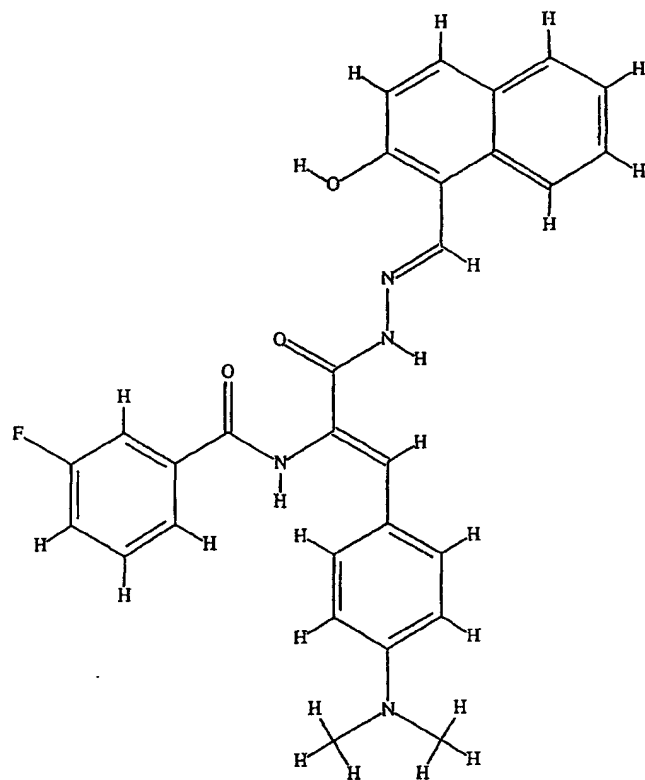


158

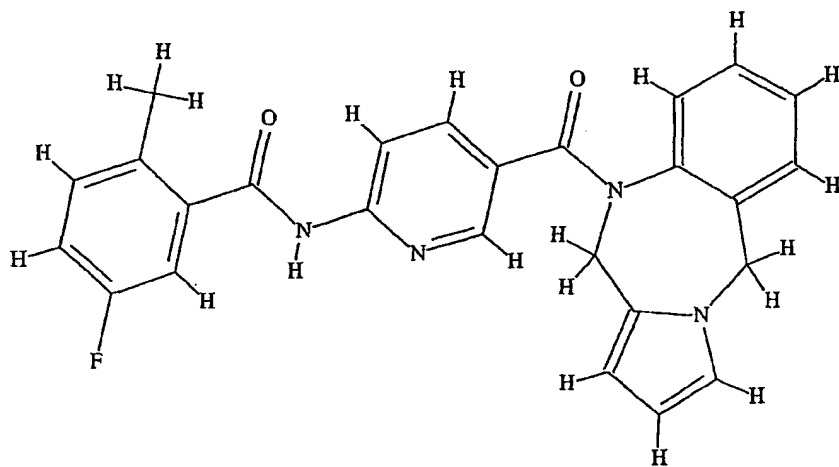


SUBSTITUTE SHEET (RULE 26)

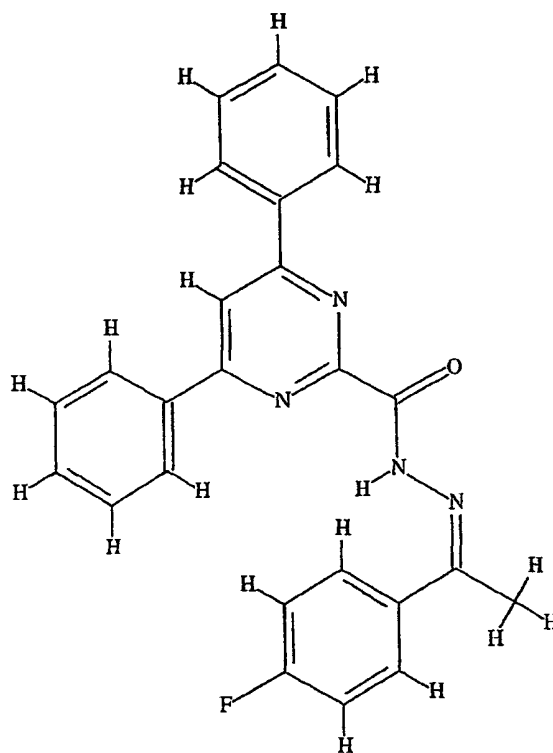
159



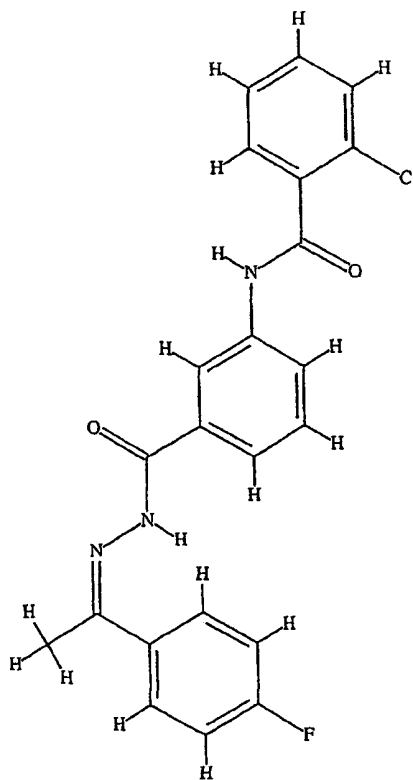
160



161



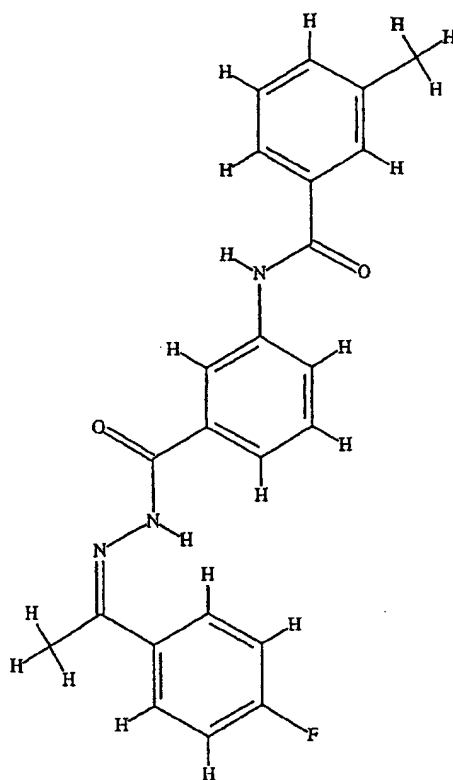
162



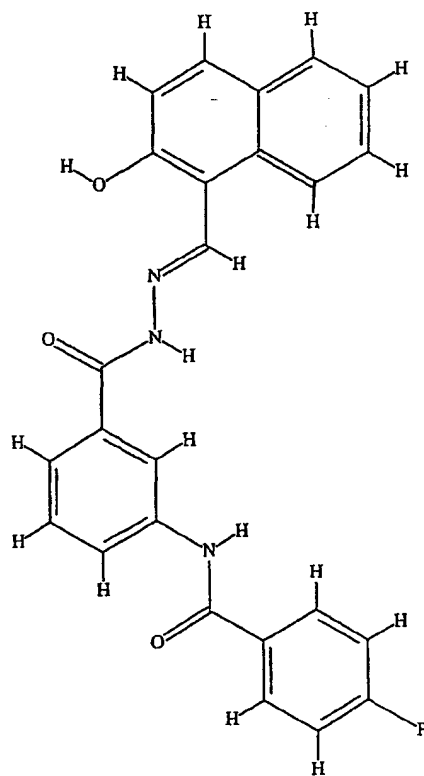
SUBSTITUTE SHEET (RULE 26)

82/248

163

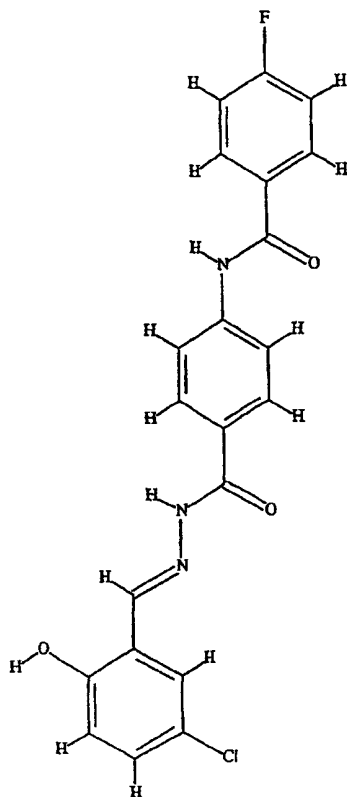


164

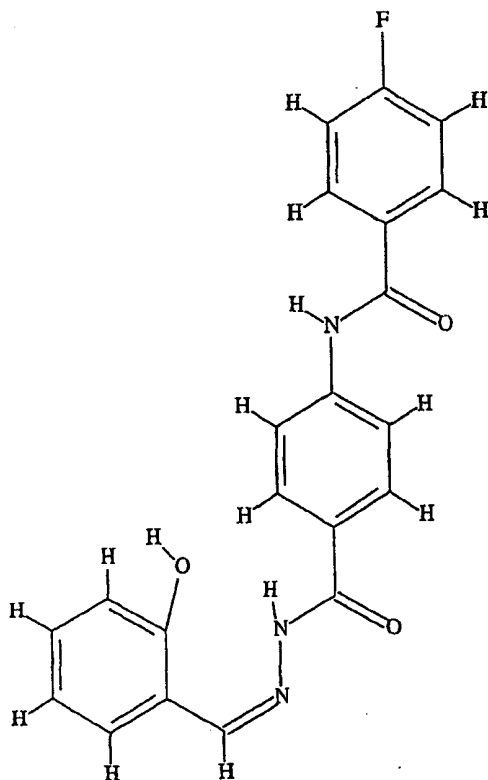


SUBSTITUTE SHEET (RULE 26)

165



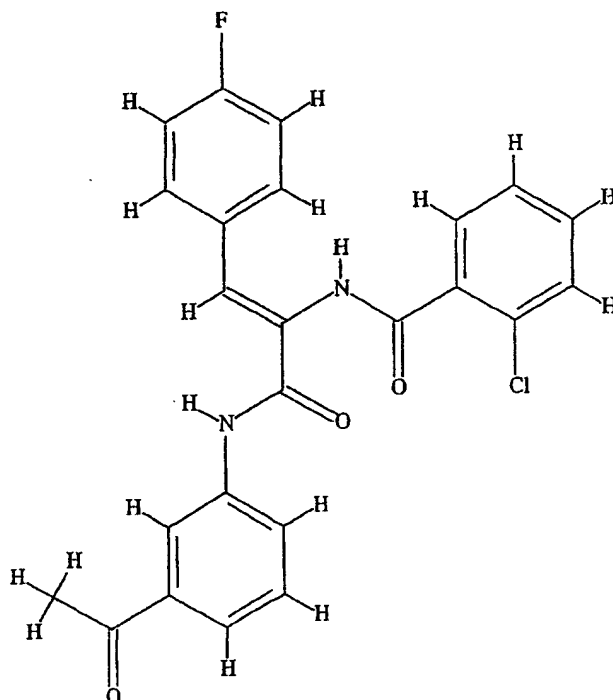
166



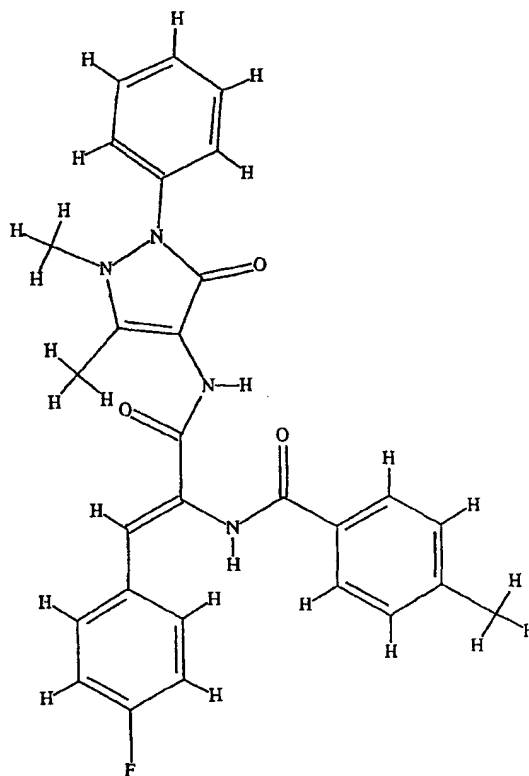
SUBSTITUTE SHEET (RULE 26)

84/248

167

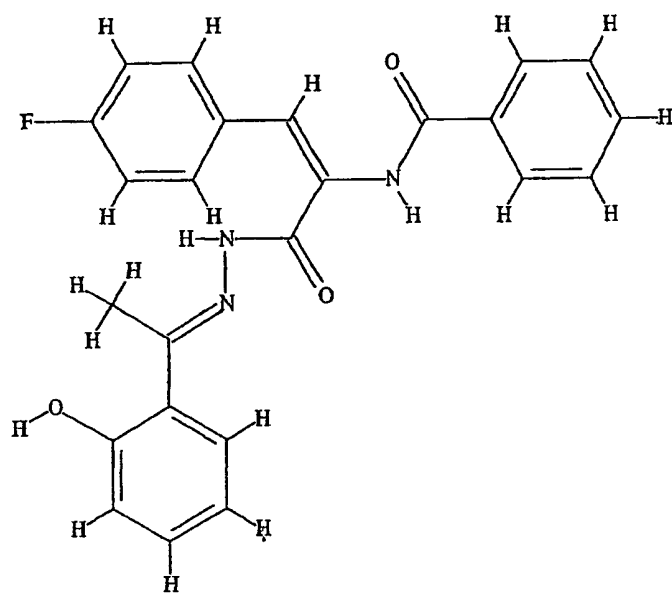


168

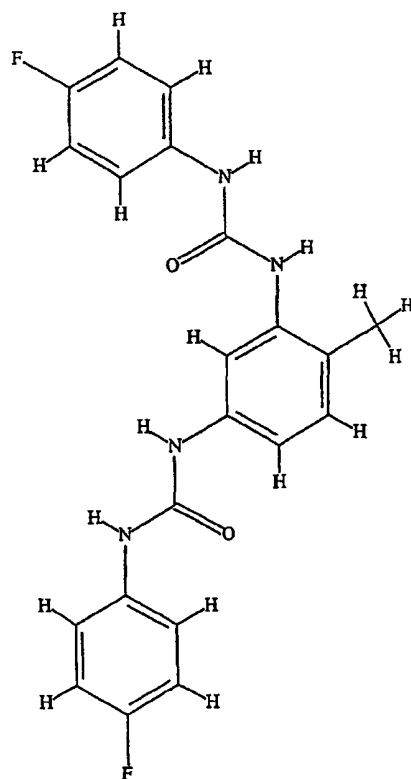


SUBSTITUTE SHEET (RULE 26)

169



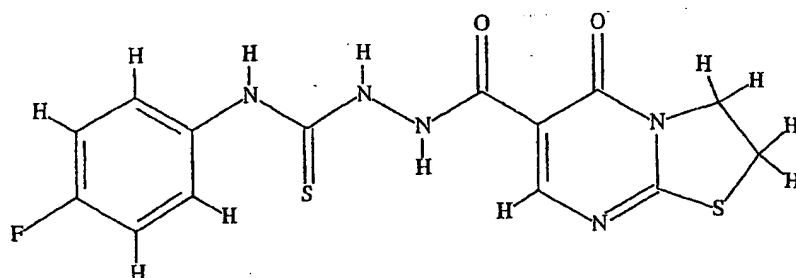
170



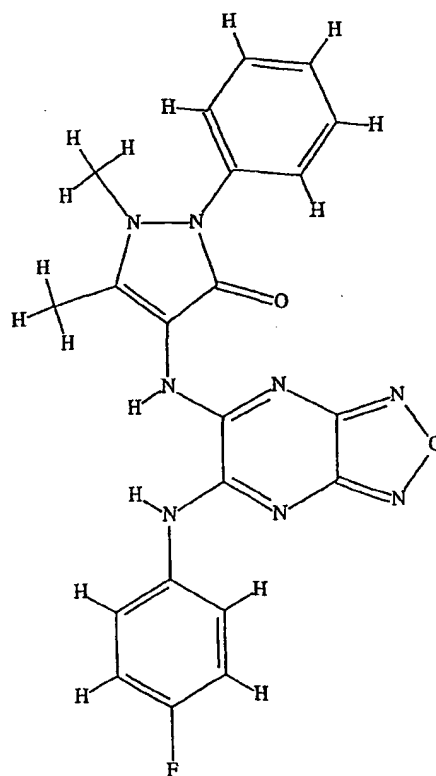
SUBSTITUTE SHEET (RULE 26)



171



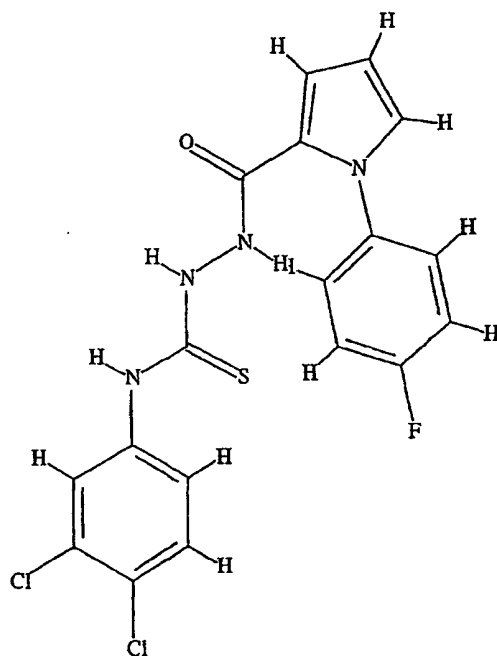
172



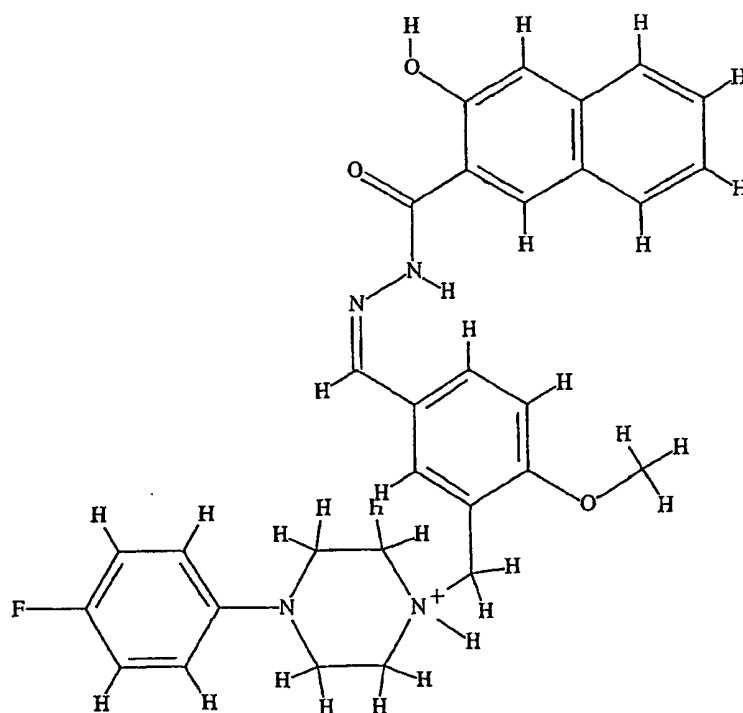
SUBSTITUTE SHEET (RULE 26)

87/248

173

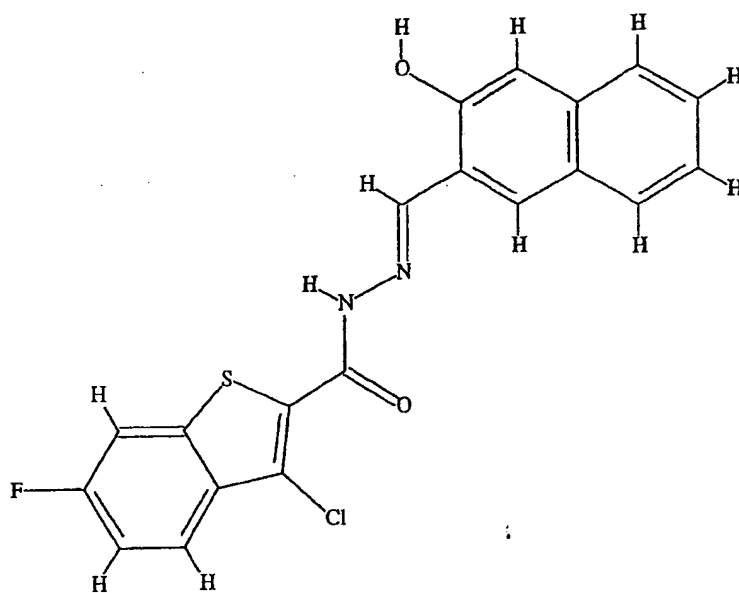


174

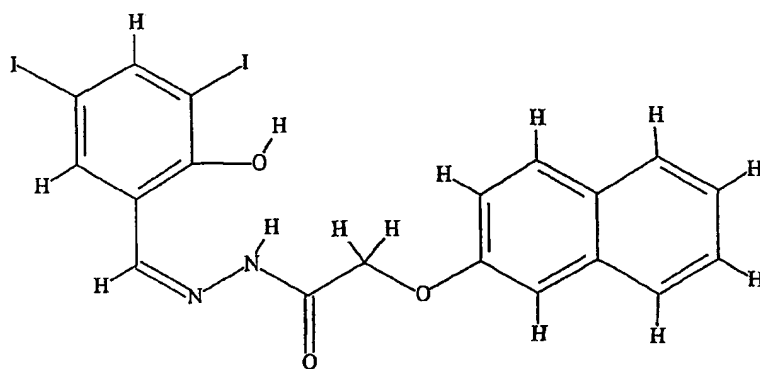


SUBSTITUTE SHEET (RULE 26)

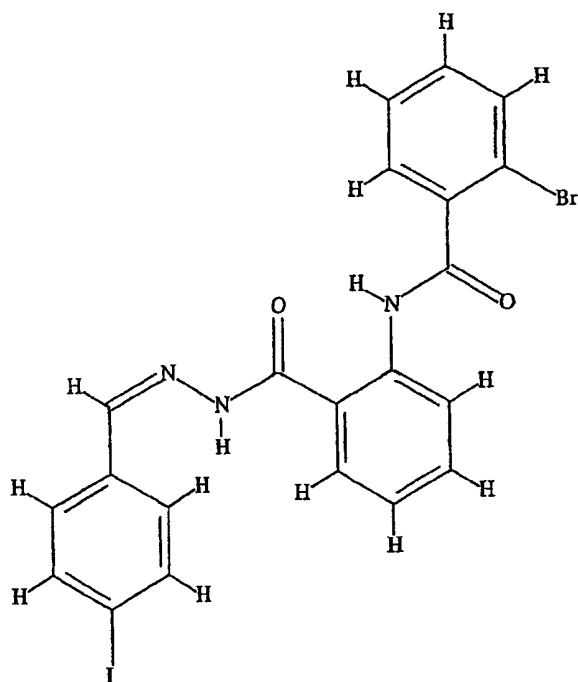
175



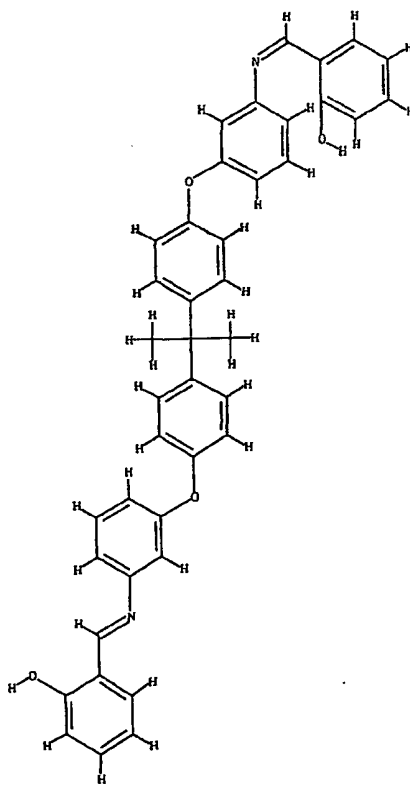
176



177



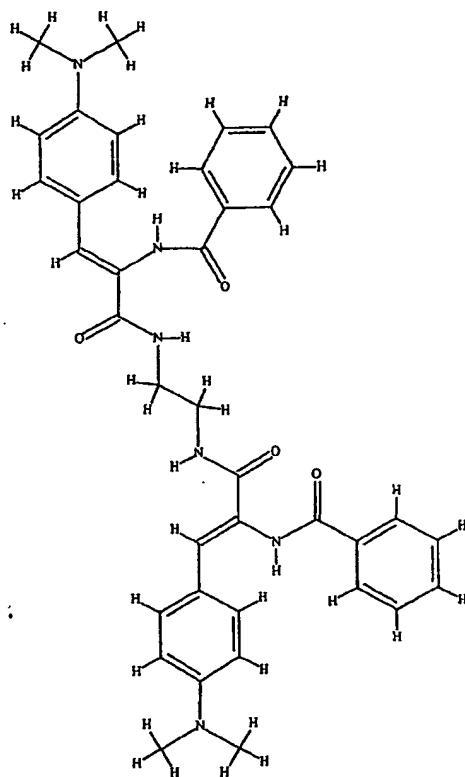
178



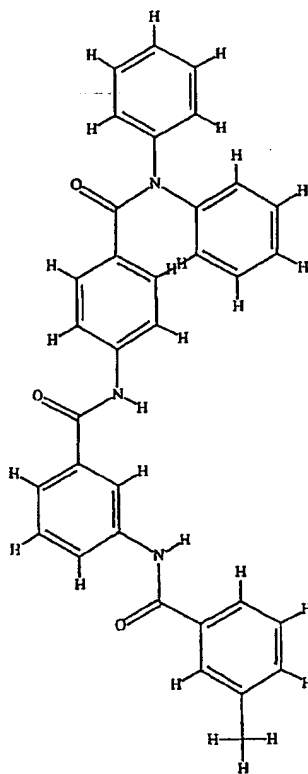
SUBSTITUTE SHEET (RULE 26)

90/248

179

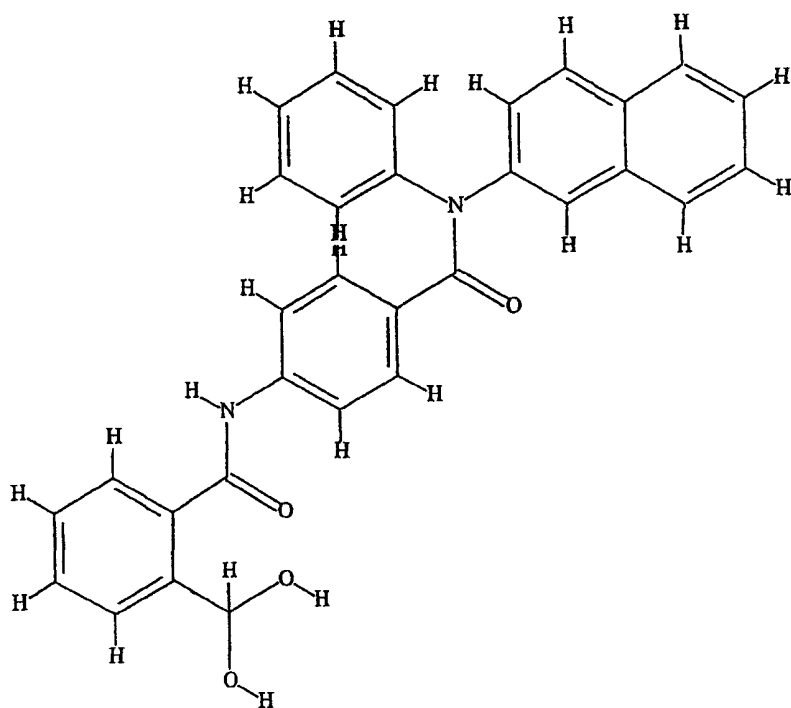


180

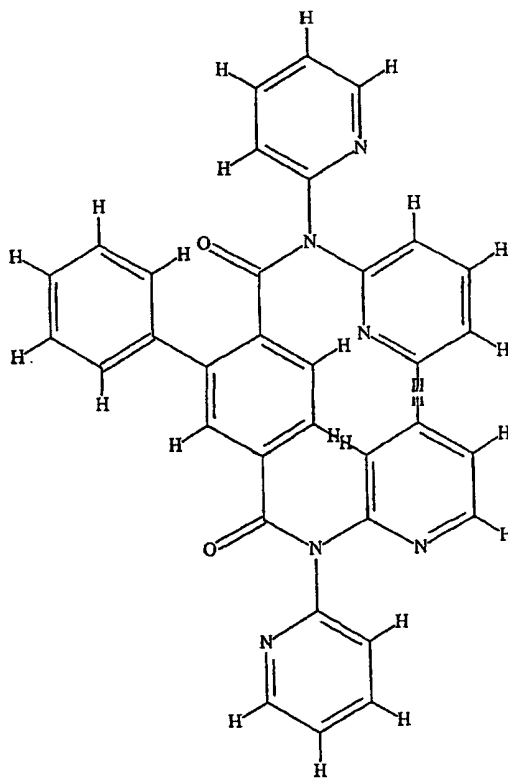


SUBSTITUTE SHEET (RULE 26)

181

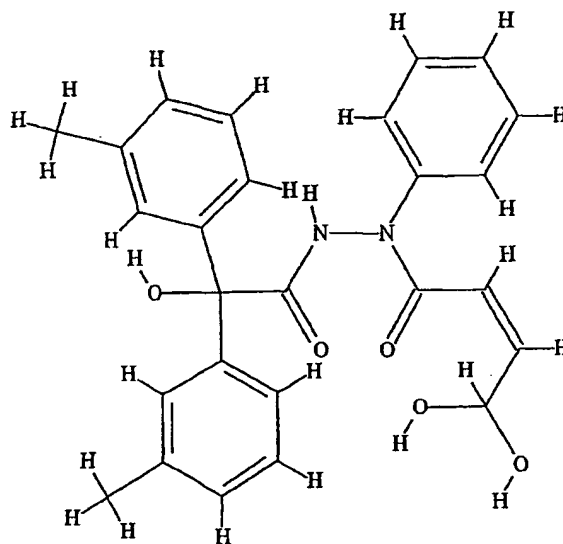


182

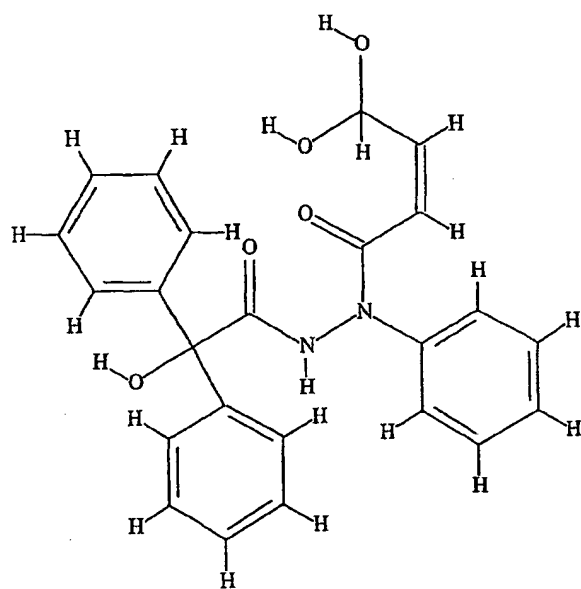


SUBSTITUTE SHEET (RULE 26)

183

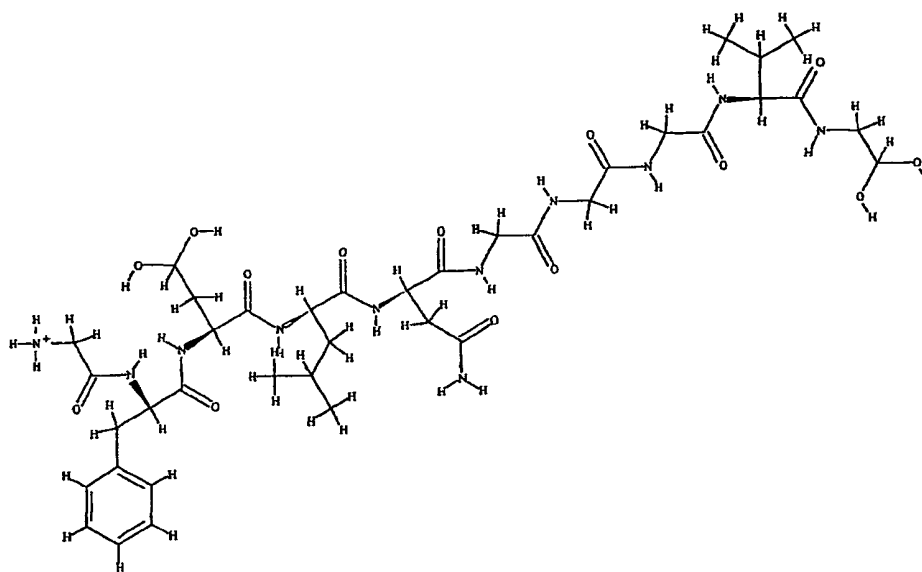


184

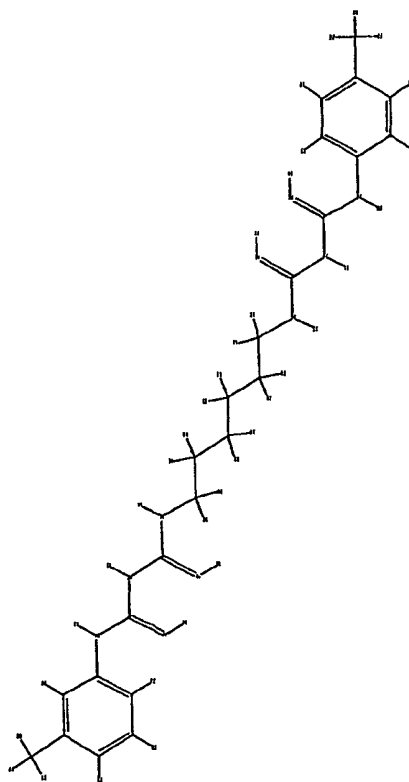


SUBSTITUTE SHEET (RULE 26)

185



186

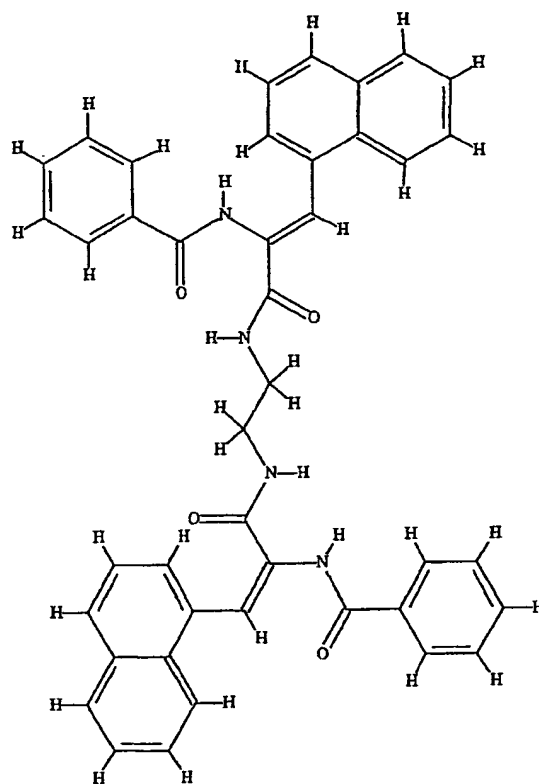


SUBSTITUTE SHEET (RULE 26)

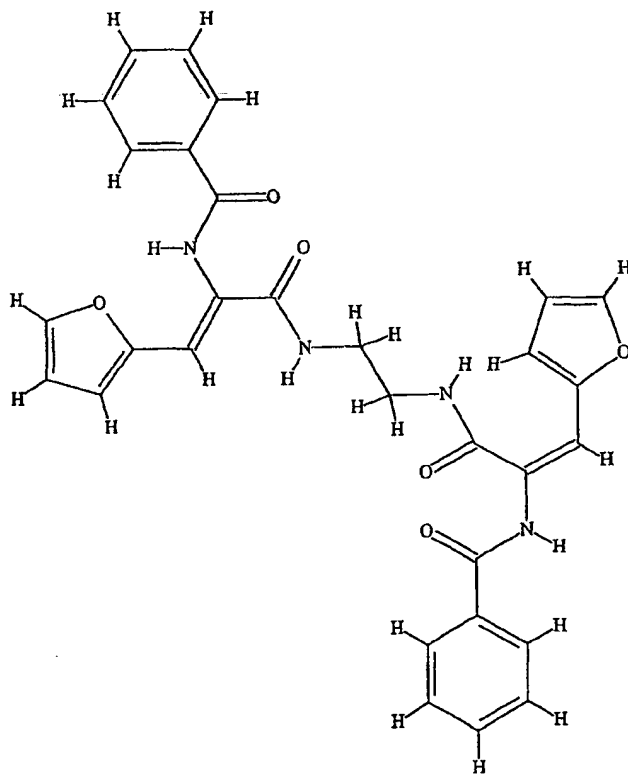


94/248

187



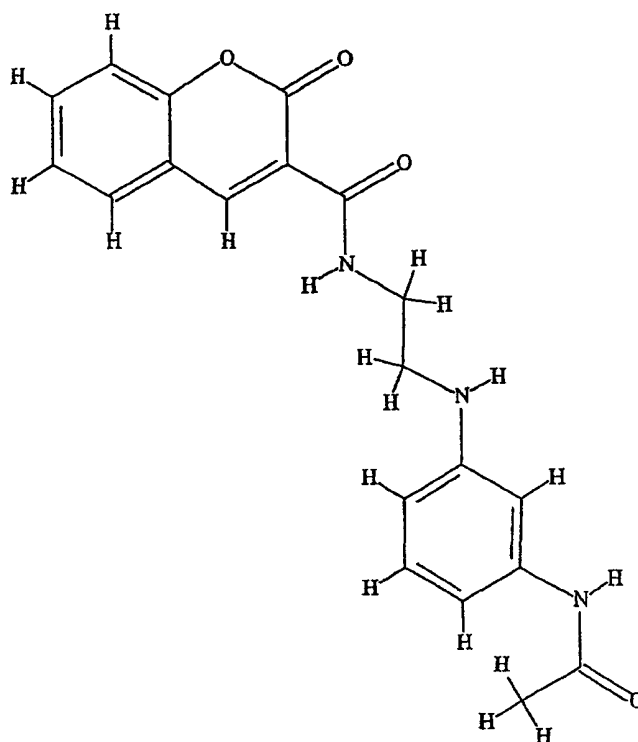
188



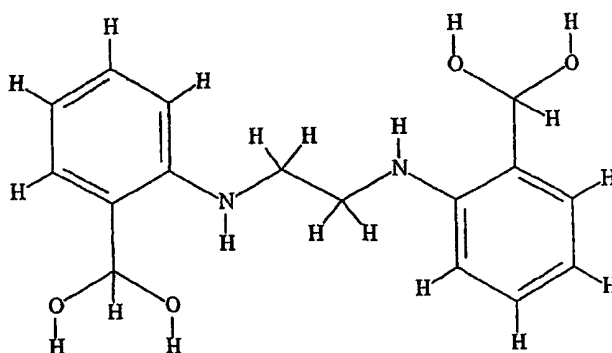
SUBSTITUTE SHEET (RULE 26)

95/248

189

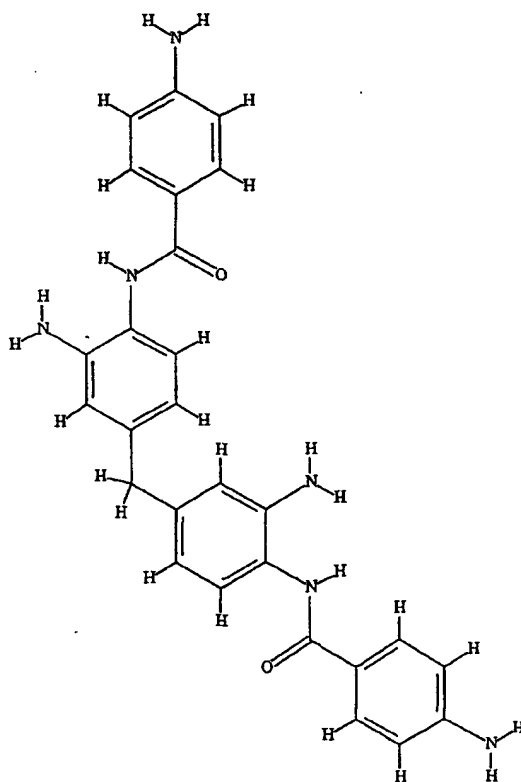


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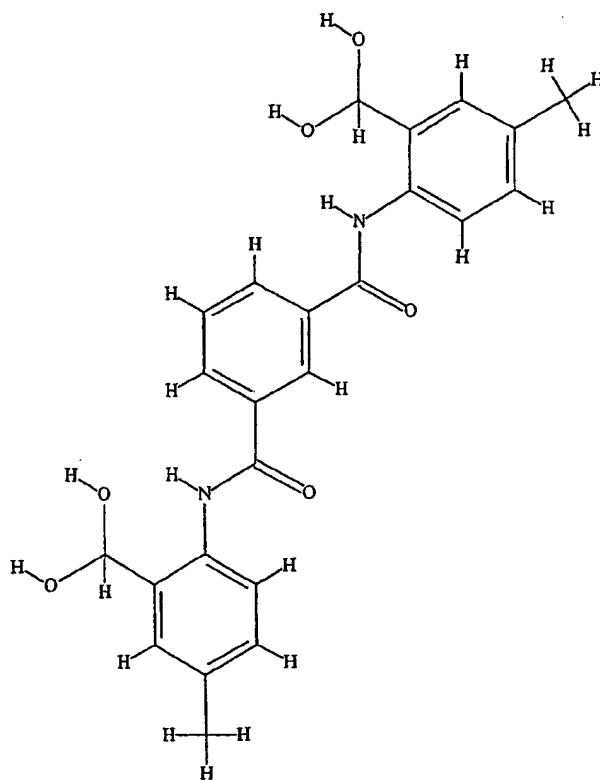


96/248

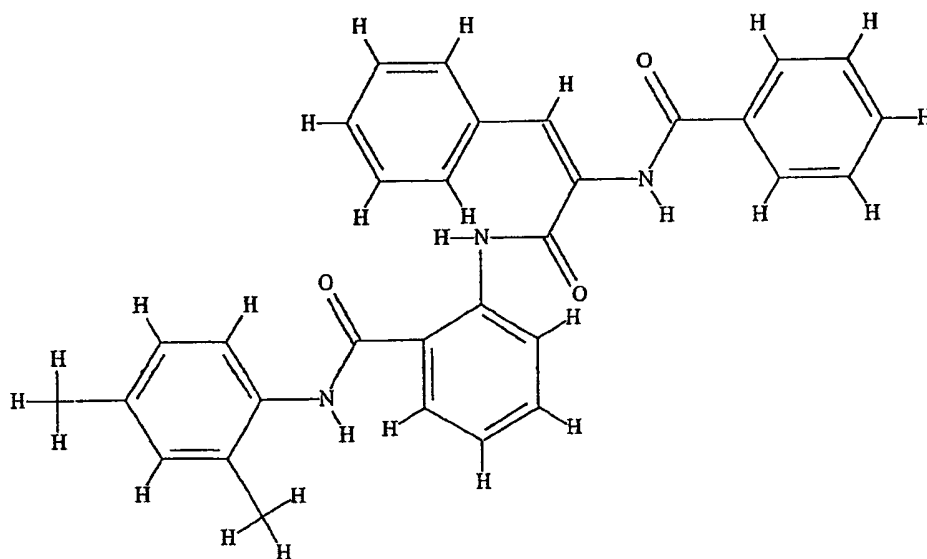
191



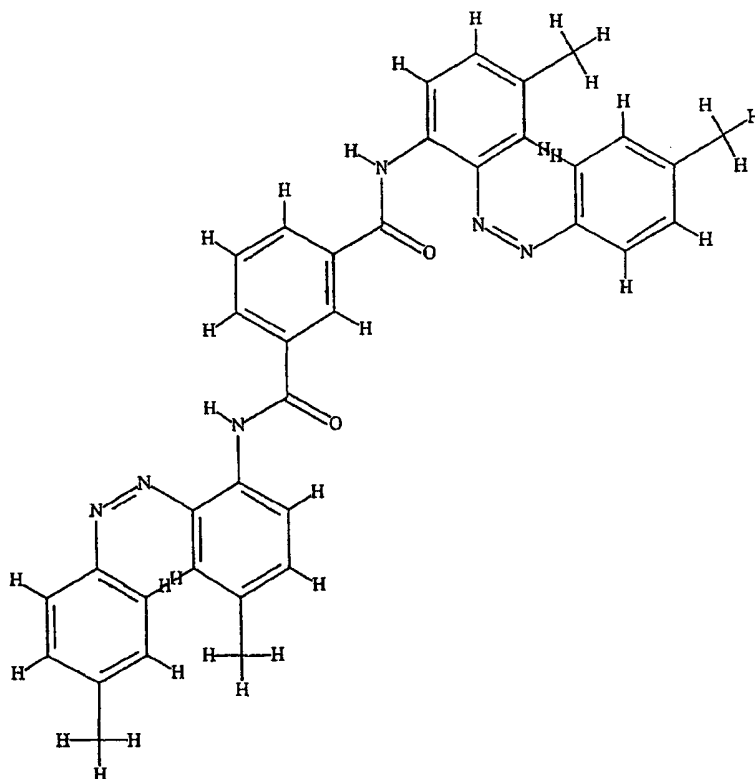
192



SUBSTITUTE SHEET (RULE 26)



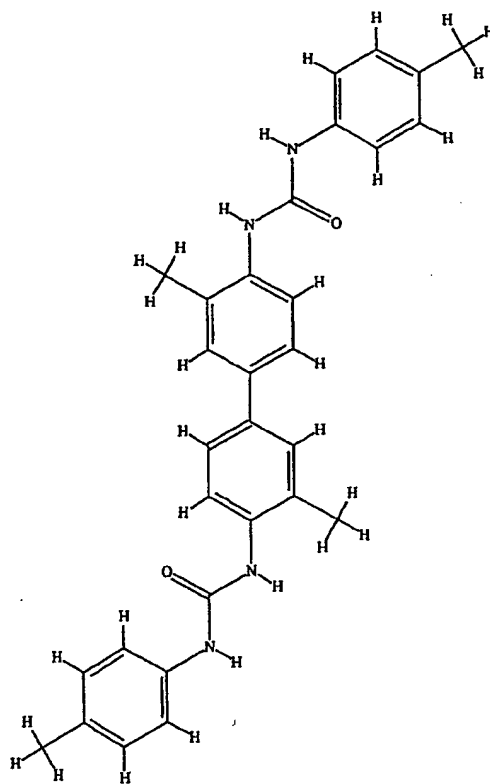
194



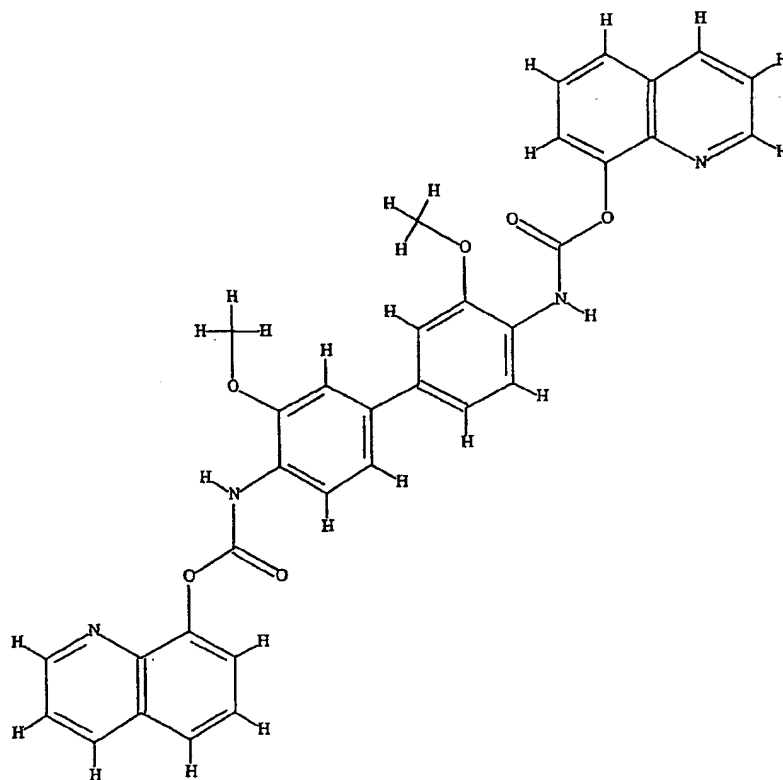
**SUBSTITUTE SHEET (RULE 26)**

98/248

195

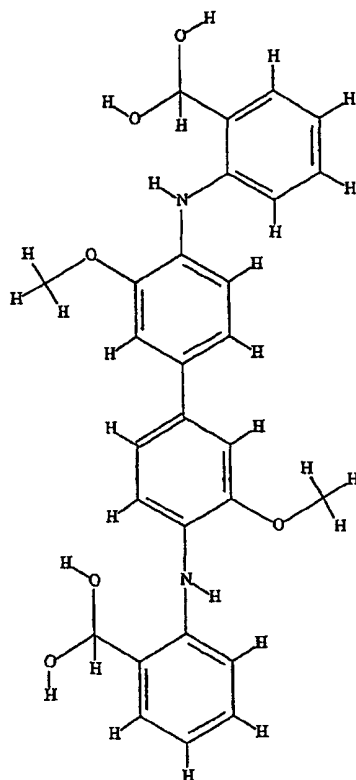


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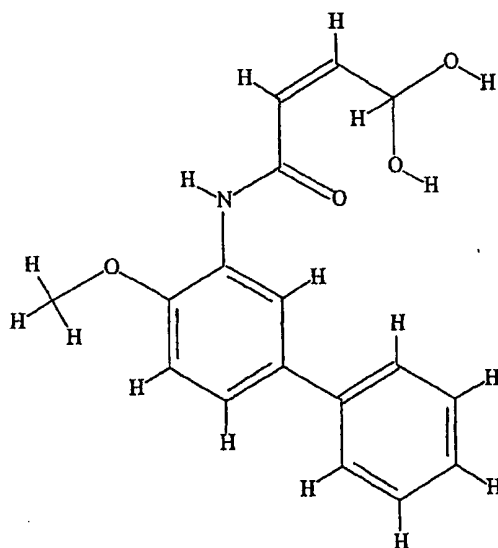


SUBSTITUTE SHEET (RULE 26)

197



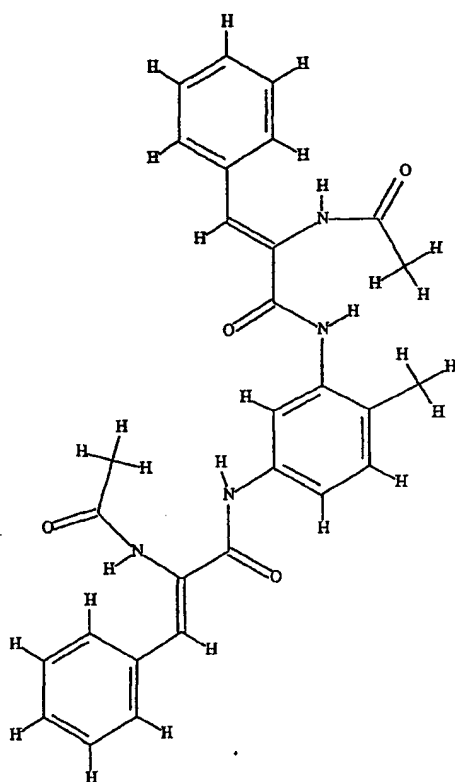
198



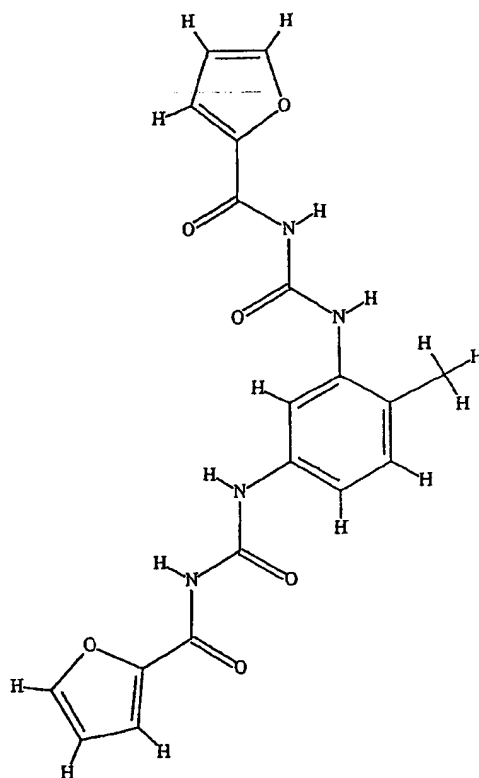
SUBSTITUTE SHEET (RULE 26)

100/248

199



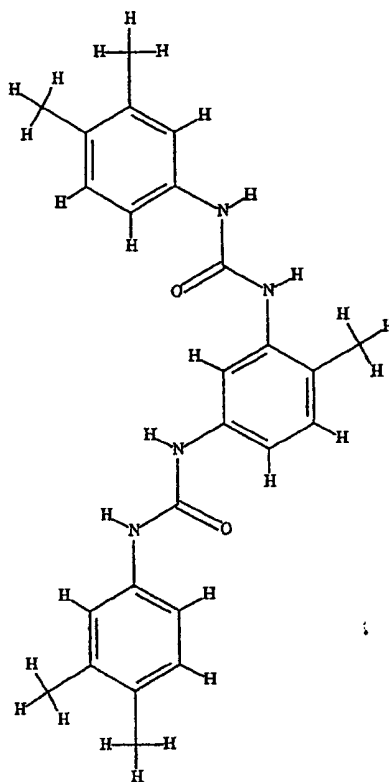
200



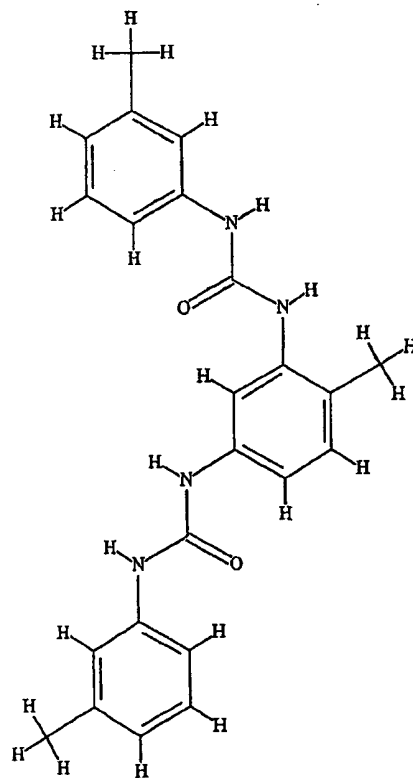
SUBSTITUTE SHEET (RULE 26)

101/248

201



202

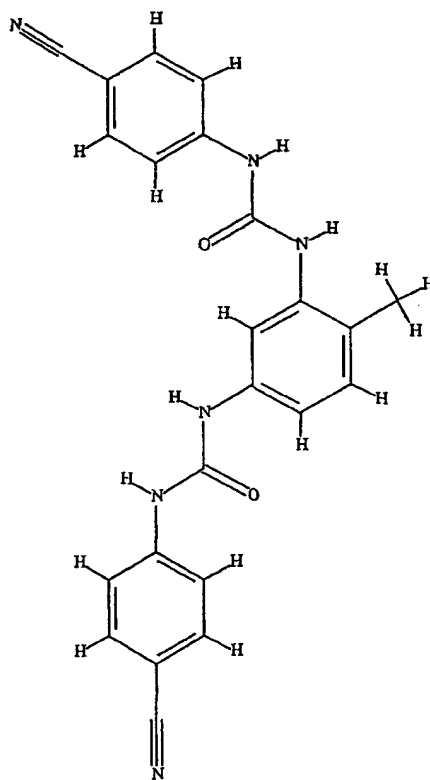


SUBSTITUTE SHEET (RULE 26)

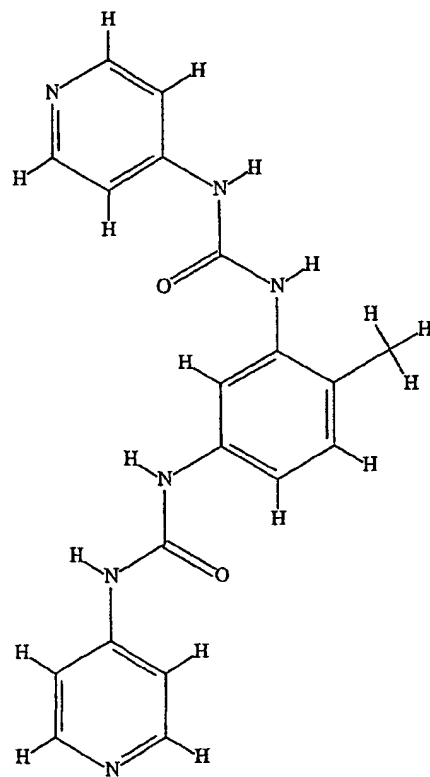


102/248

203

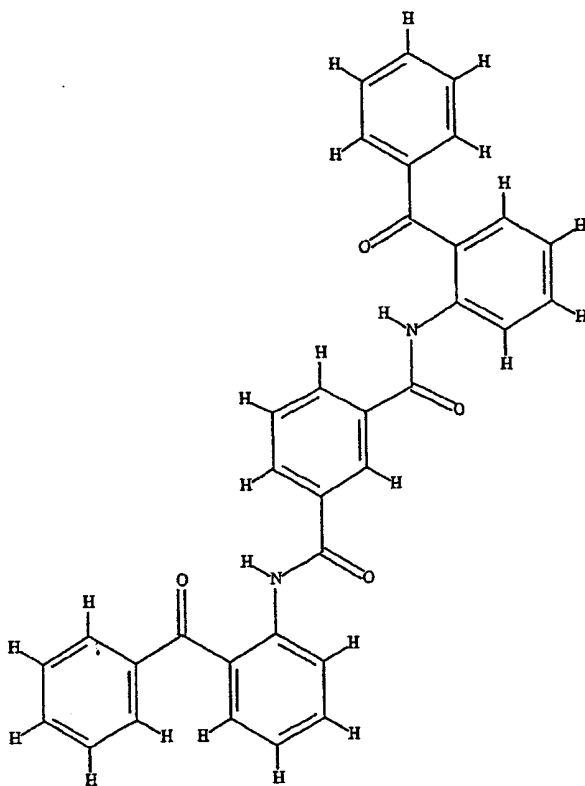


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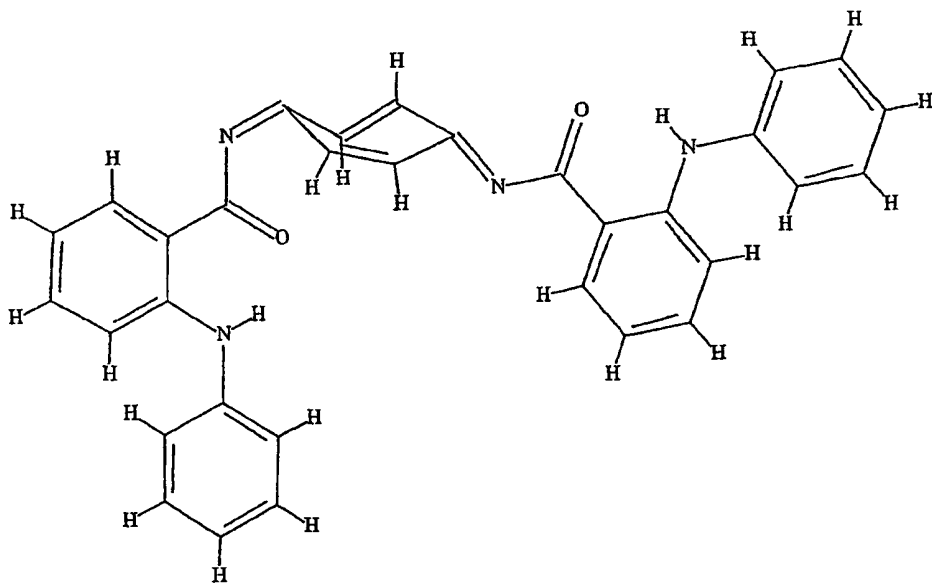


SUBSTITUTE SHEET (RULE 26)

205

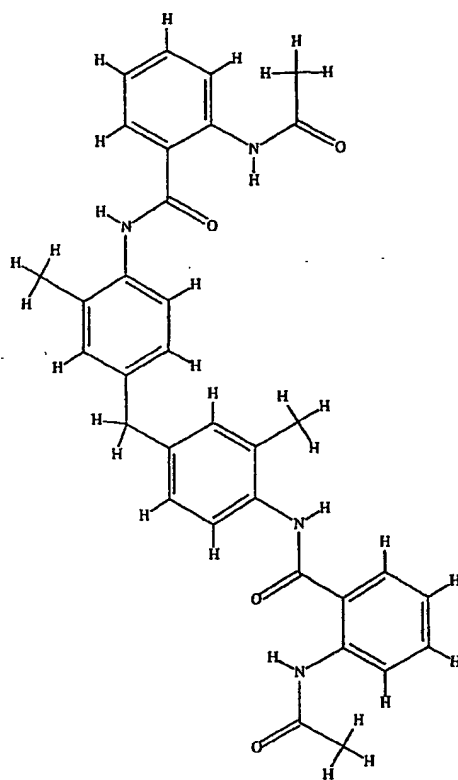


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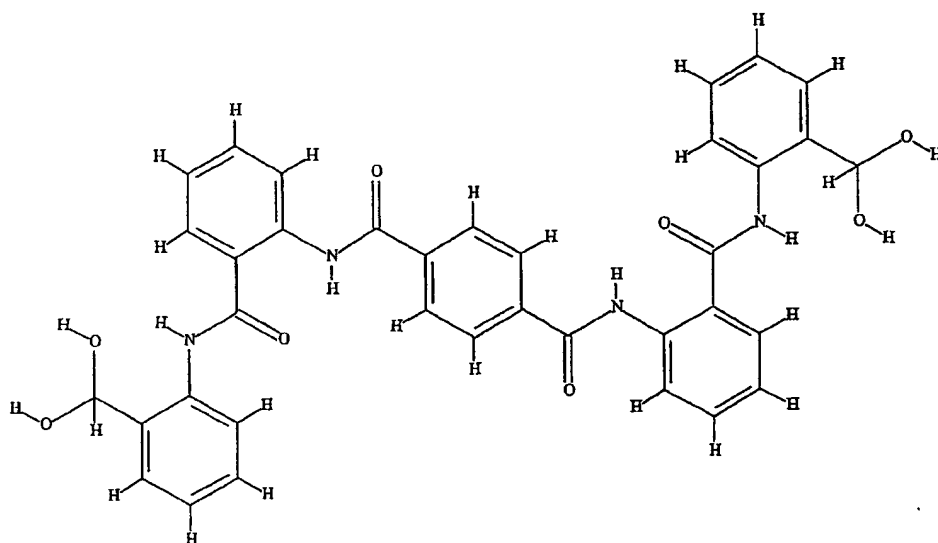


104/248

207

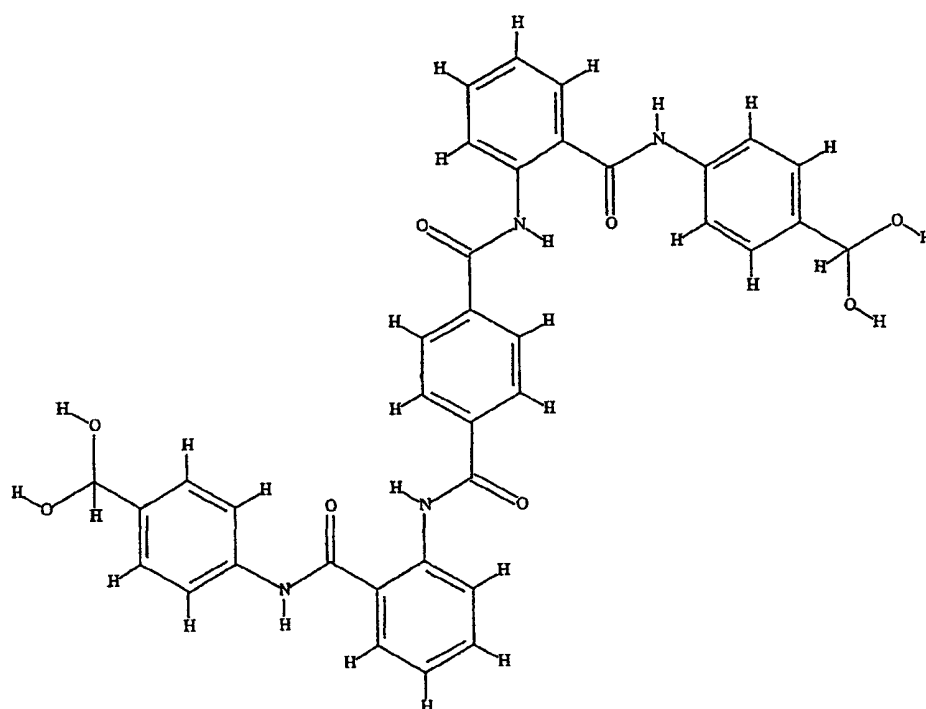


208

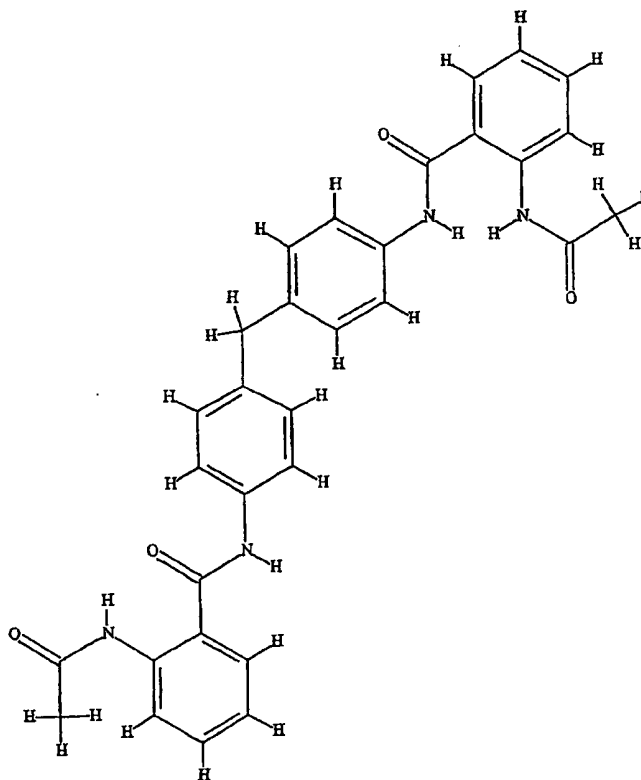


SUBSTITUTE SHEET (RULE 26)

209

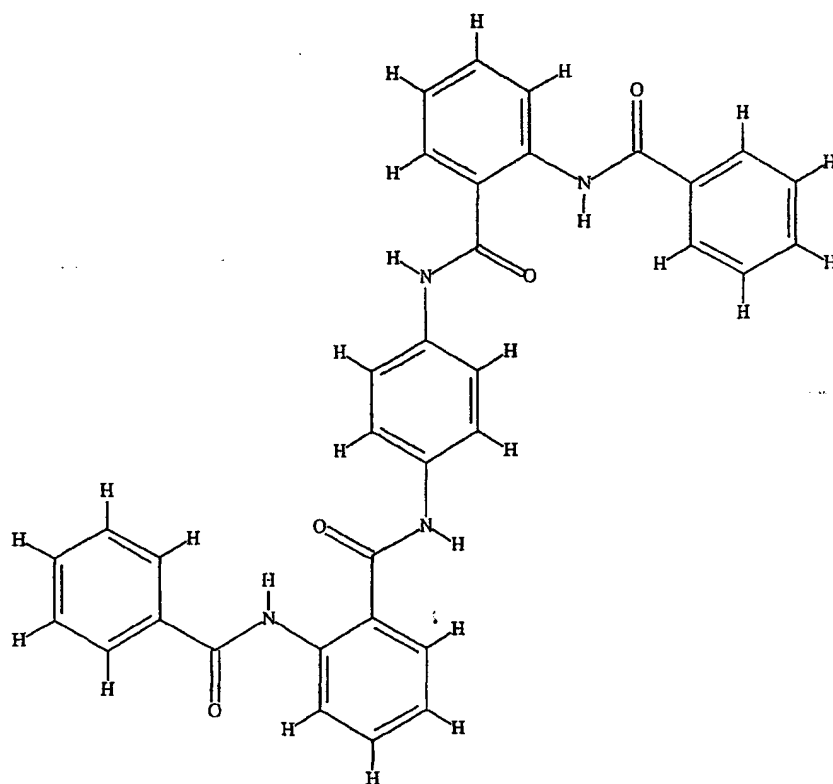


210

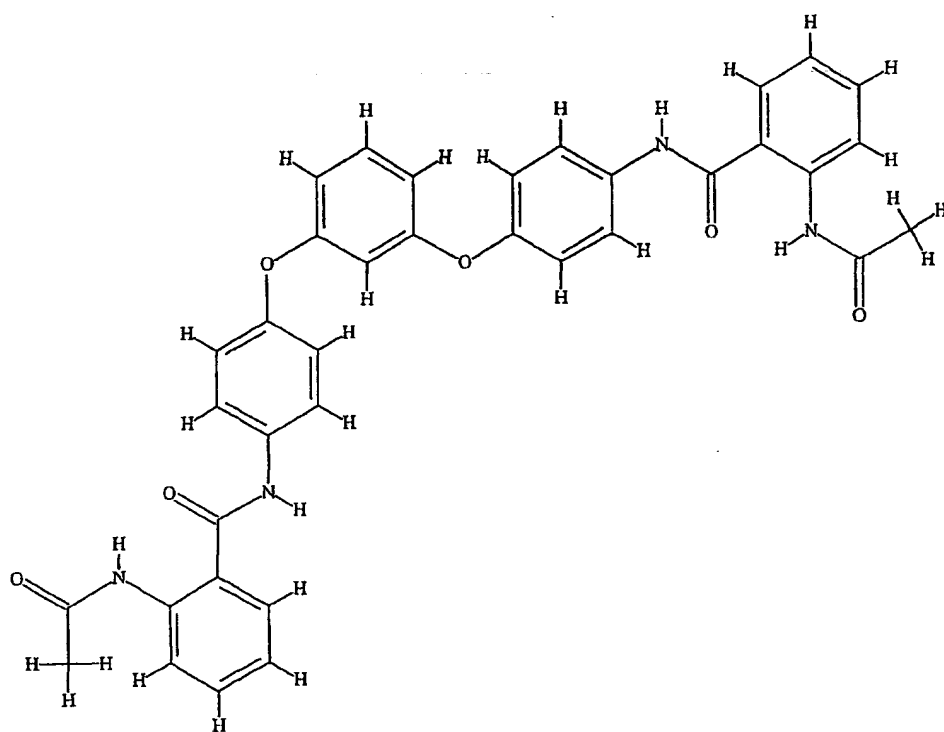


SUBSTITUTE SHEET (RULE 26)

211



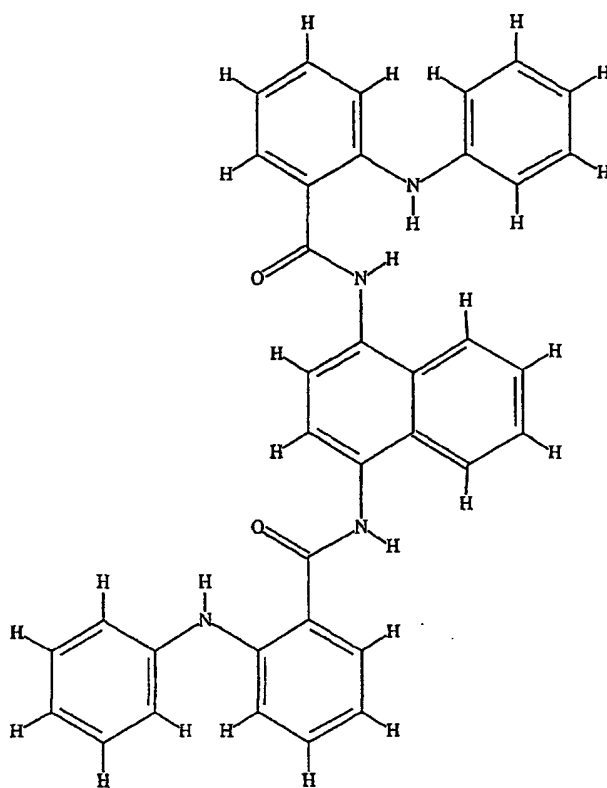
212



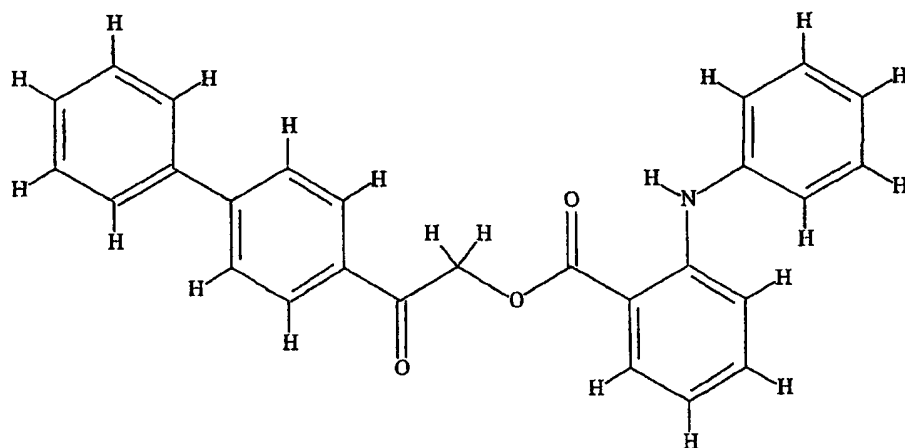
SUBSTITUTE SHEET (RULE 26)

107/248

213



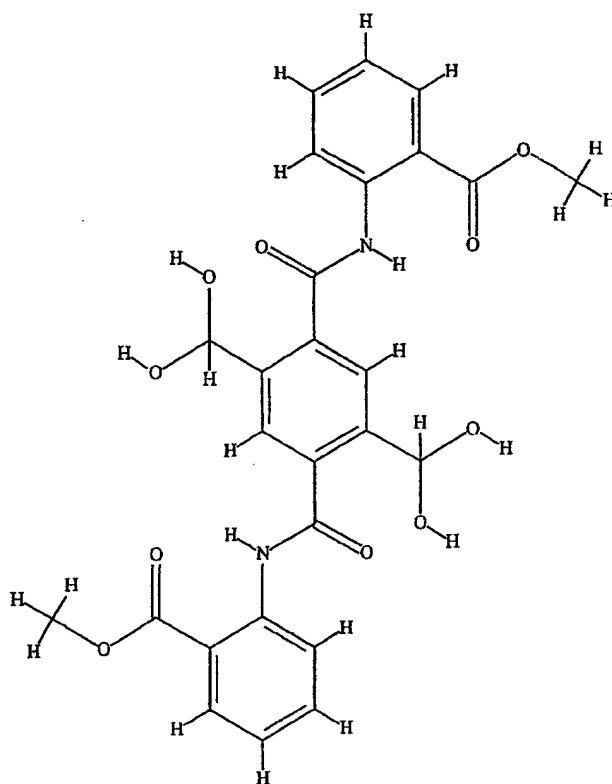
214



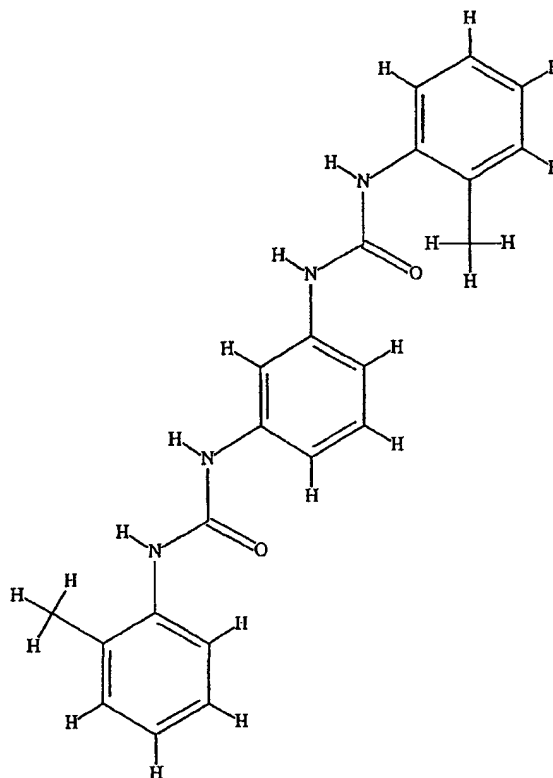
SUBSTITUTE SHEET (RULE 26)

108/248

215

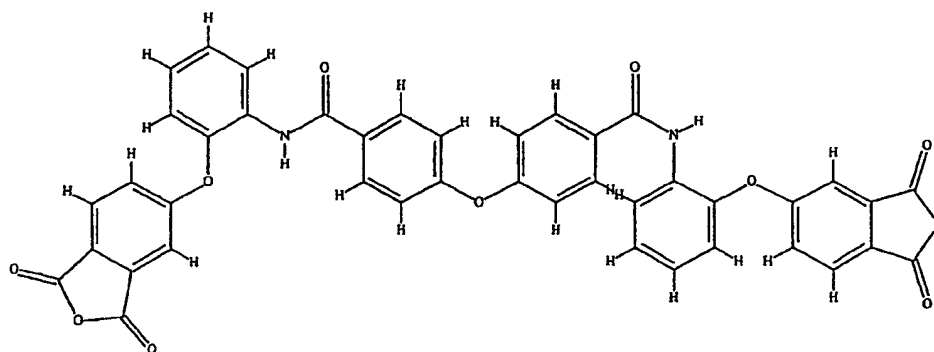


216

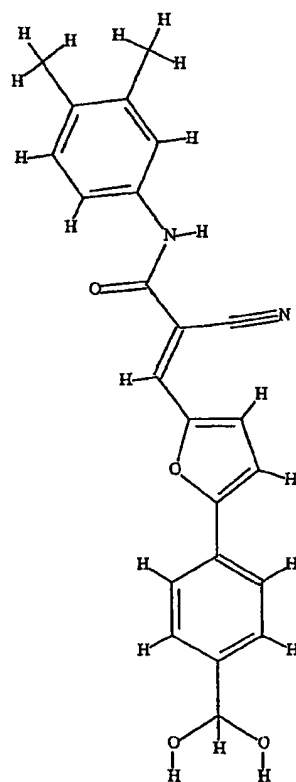


SUBSTITUTE SHEET (RULE 26)

217

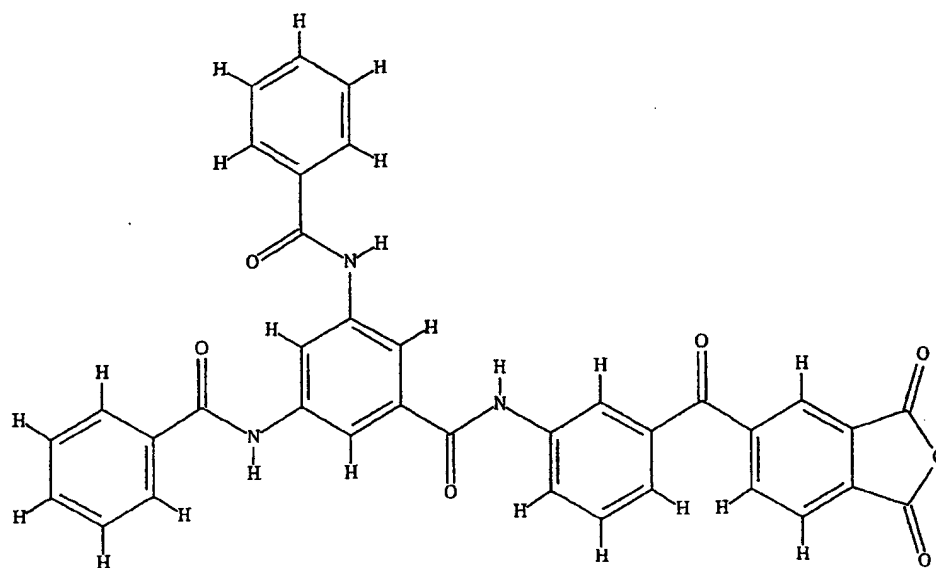


218

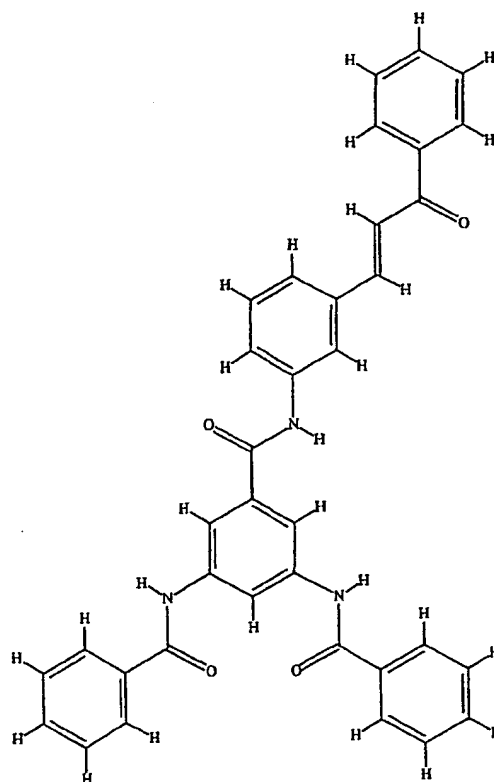


SUBSTITUTE SHEET (RULE 26)



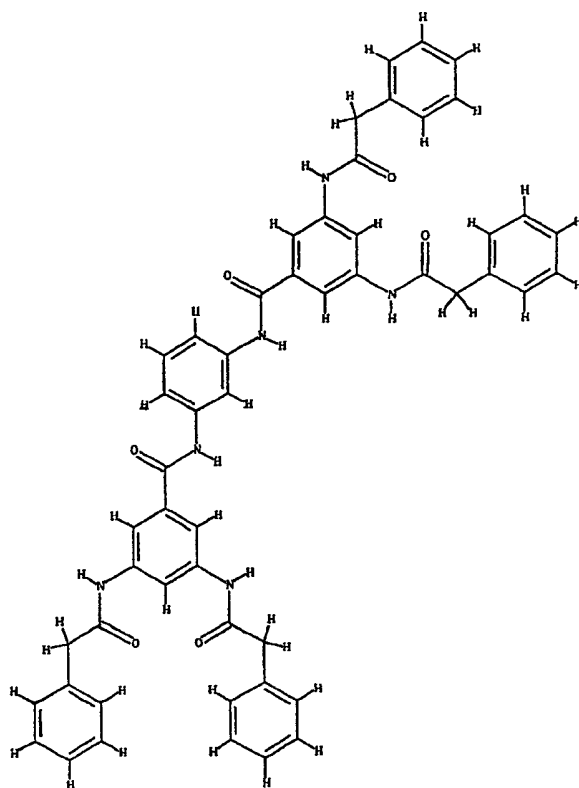


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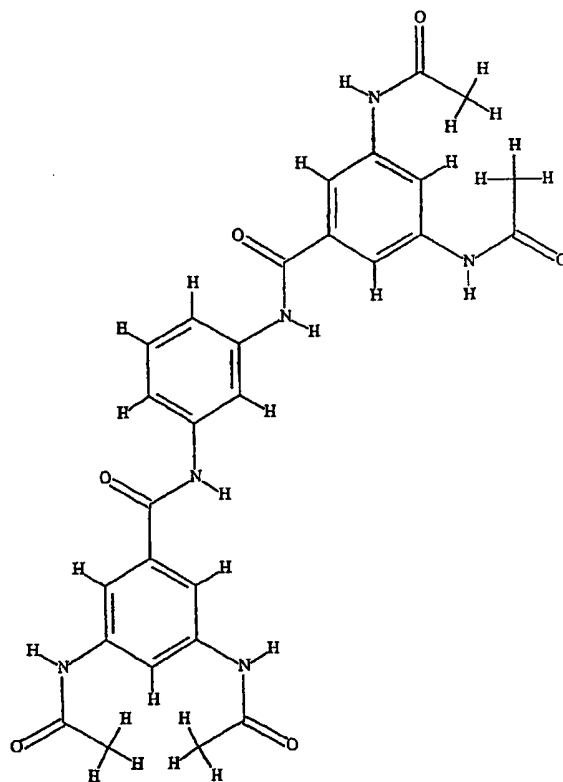


**SUBSTITUTE SHEET (RULE 26)**

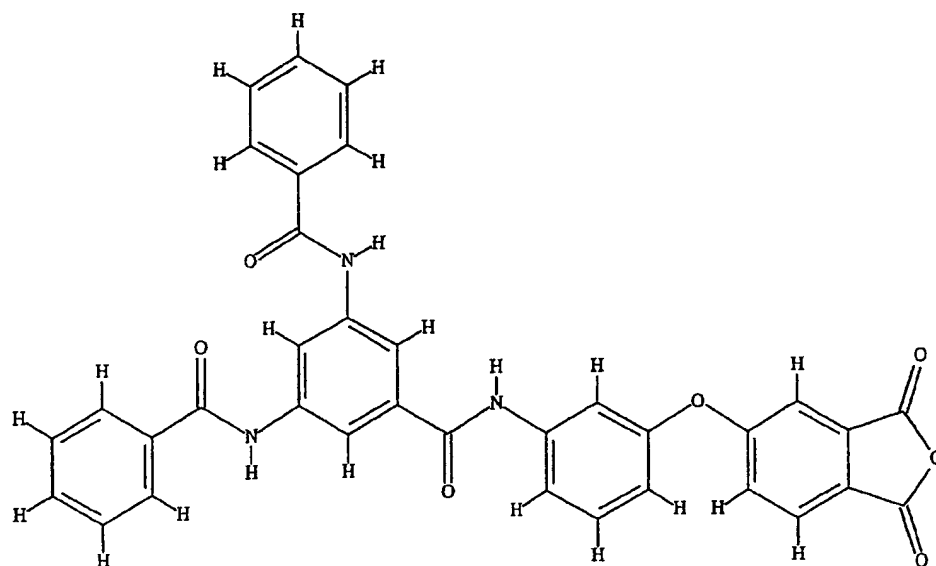
221



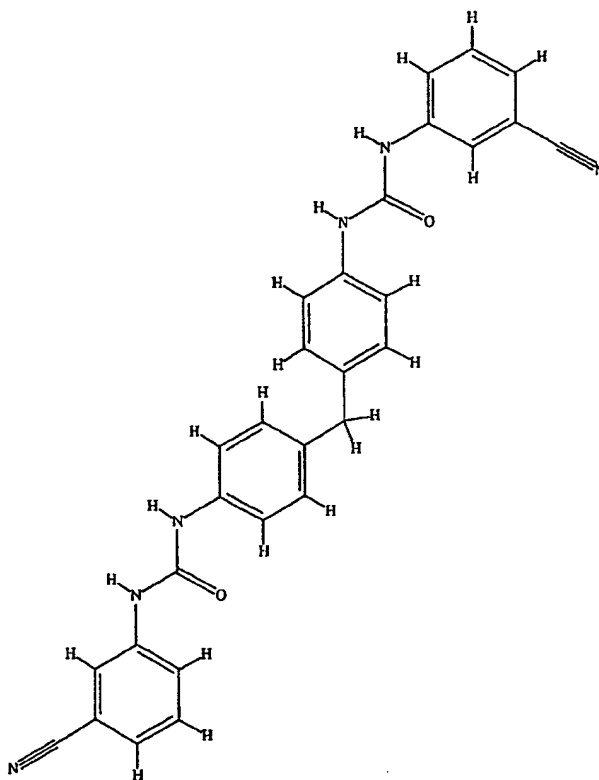
222



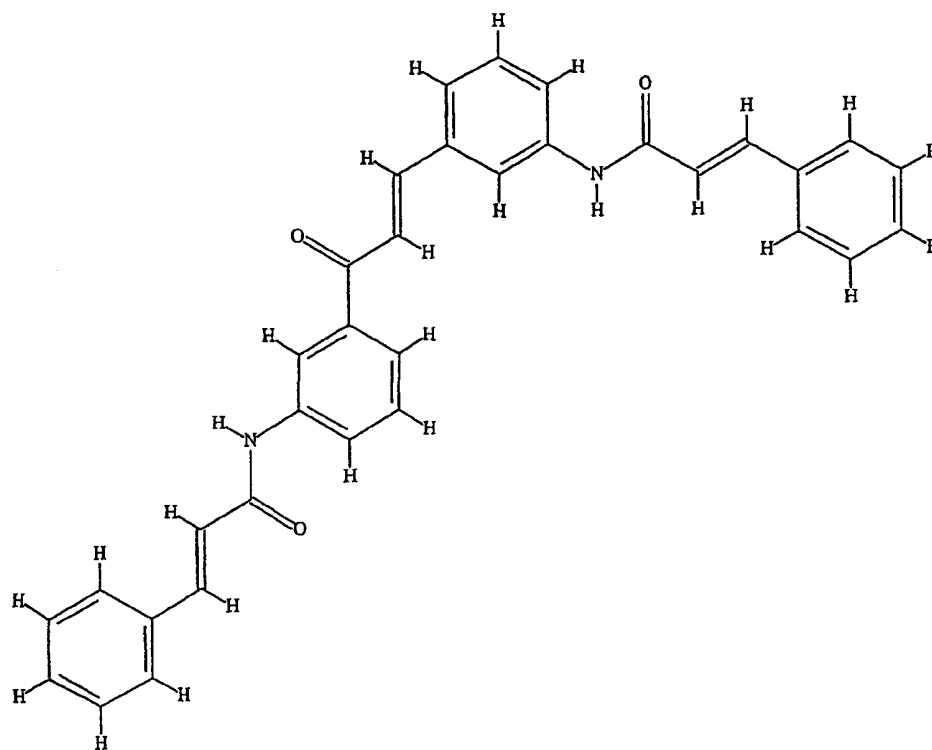
SUBSTITUTE SHEET (RULE 26)



224



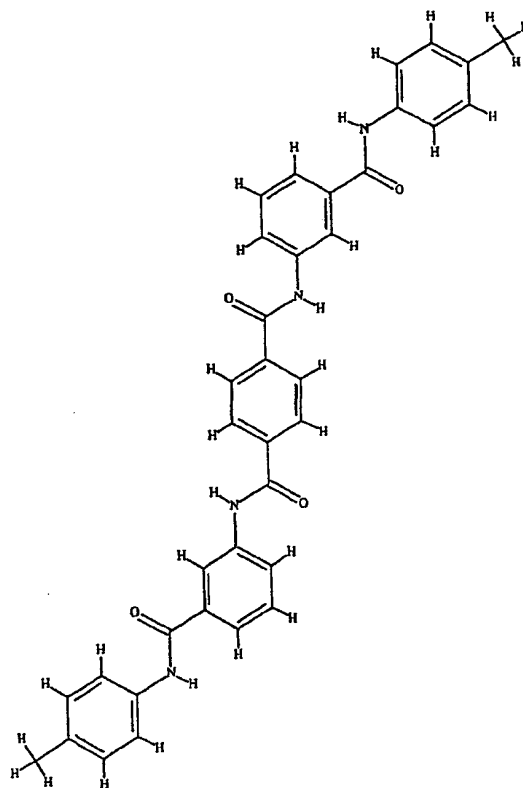
**SUBSTITUTE SHEET (RULE 26)**



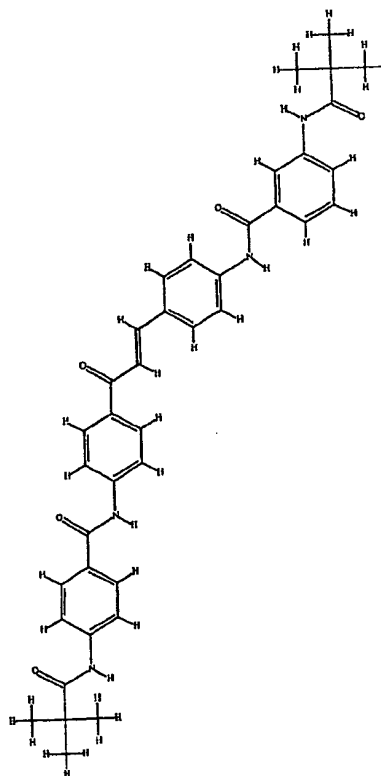
The chemical structure represents 1,3-bis(4-formylphenyl)-5-((4-formylphenyl)amino)benzene. It features a central benzene ring with an amino group (-NH-) at position 1 and two formyl groups (-CHO) at positions 3 and 5. Each formyl group is connected to a 4-aminophenyl ring, which is in turn connected to a 4-formylphenyl ring. The structure is shown in a zig-zag conformation.

BNSDOCID: <WO\_\_\_\_\_0168122A2\_1\_>

227

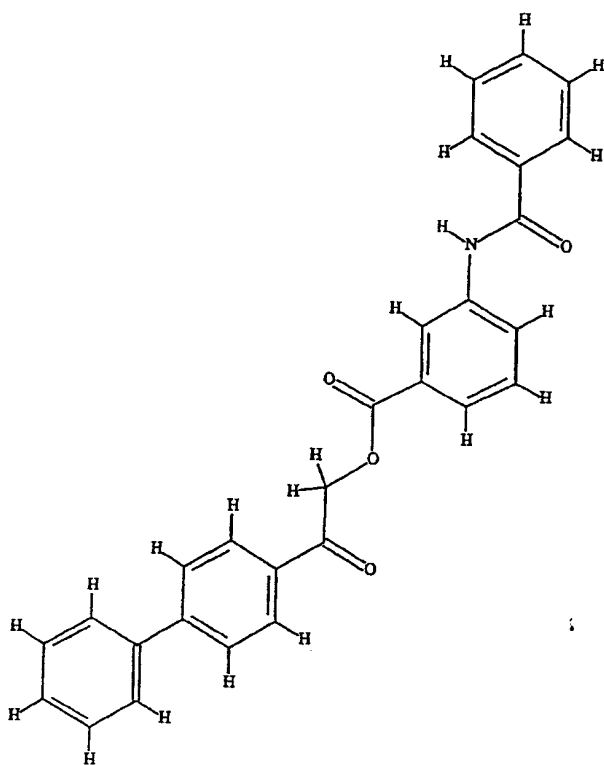


228

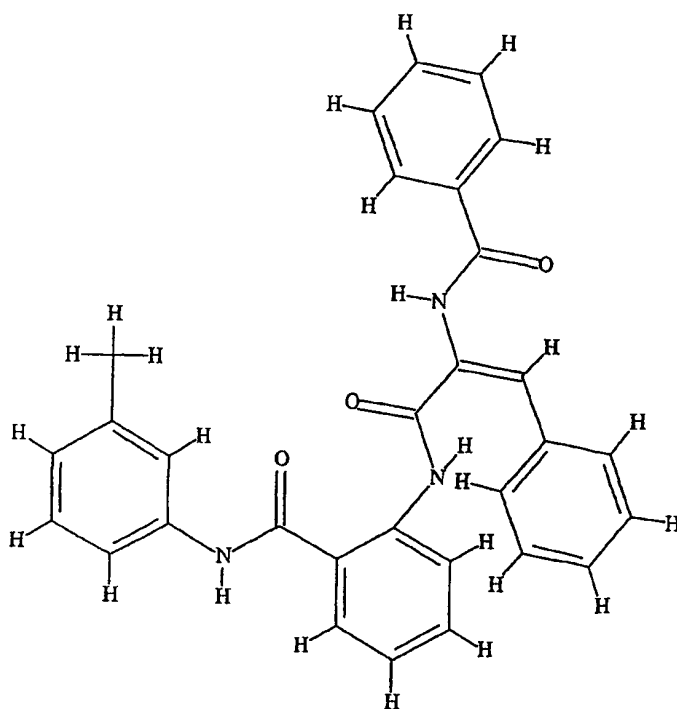


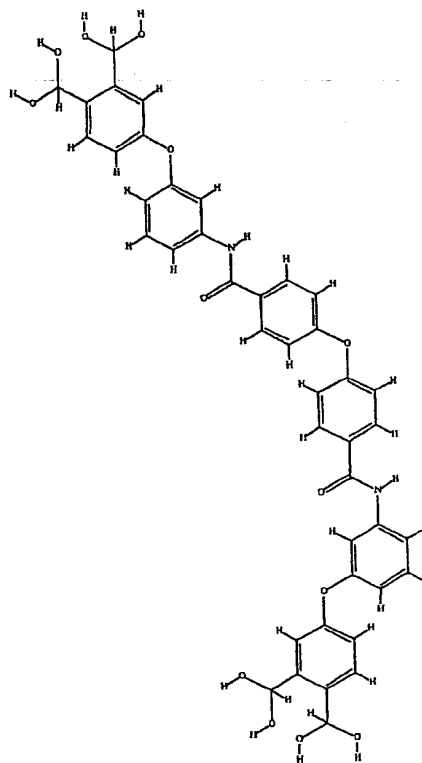
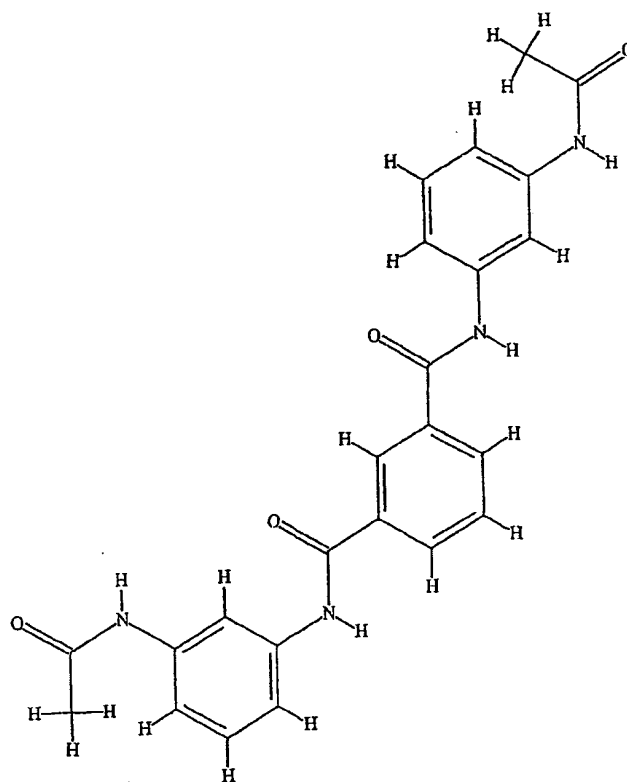
SUBSTITUTE SHEET (RULE 26)

229



230

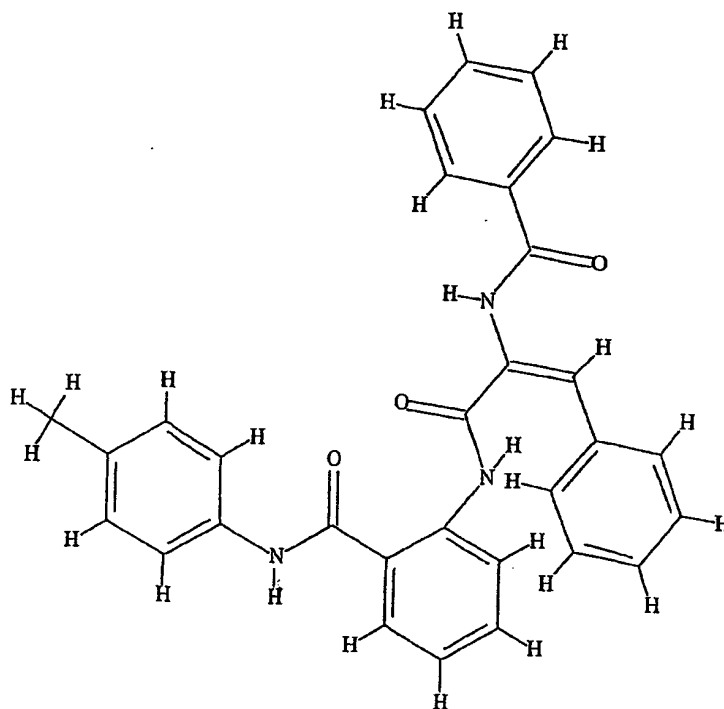




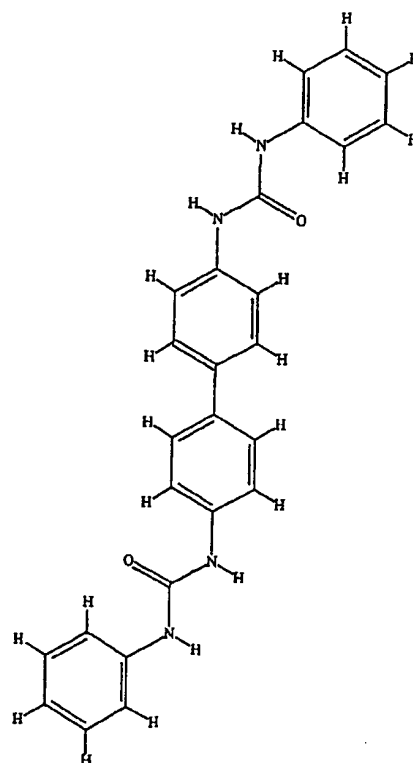
232

**SUBSTITUTE SHEET (RULE 26)**

233



234

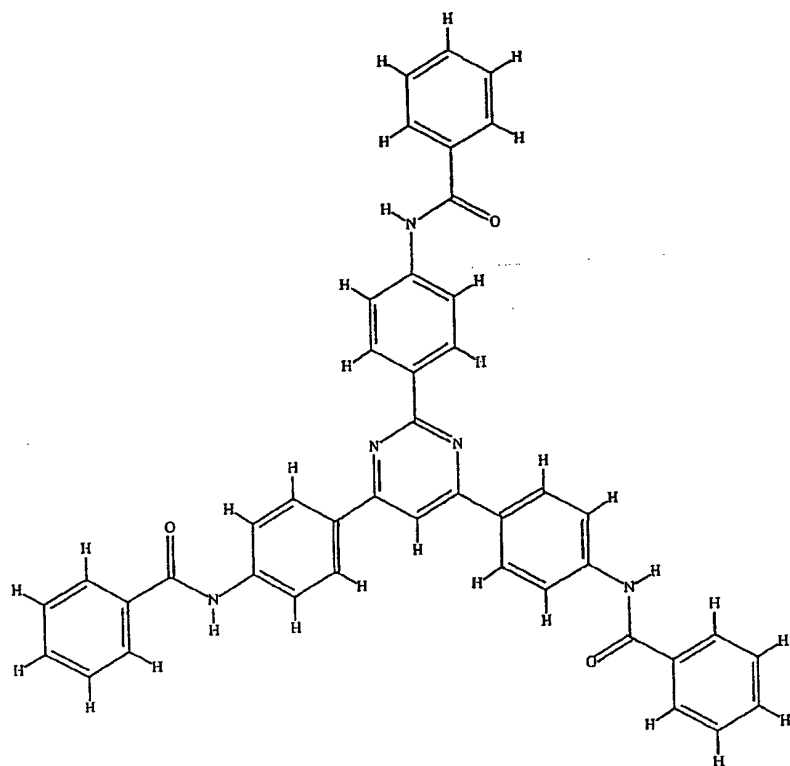


SUBSTITUTE SHEET (RULE 26)

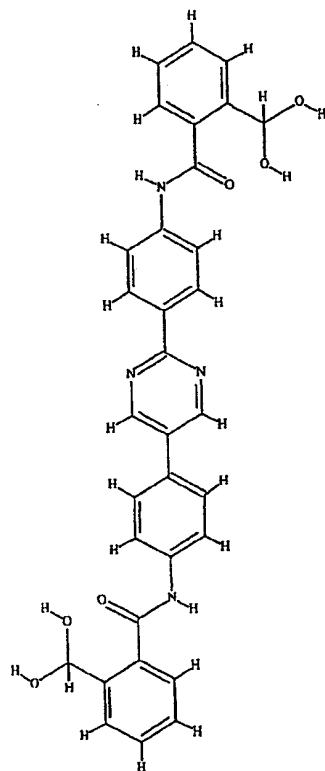


118/248

235



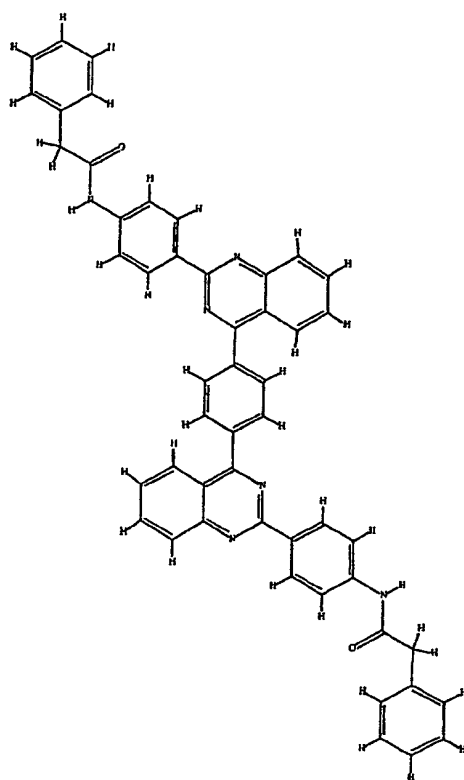
236



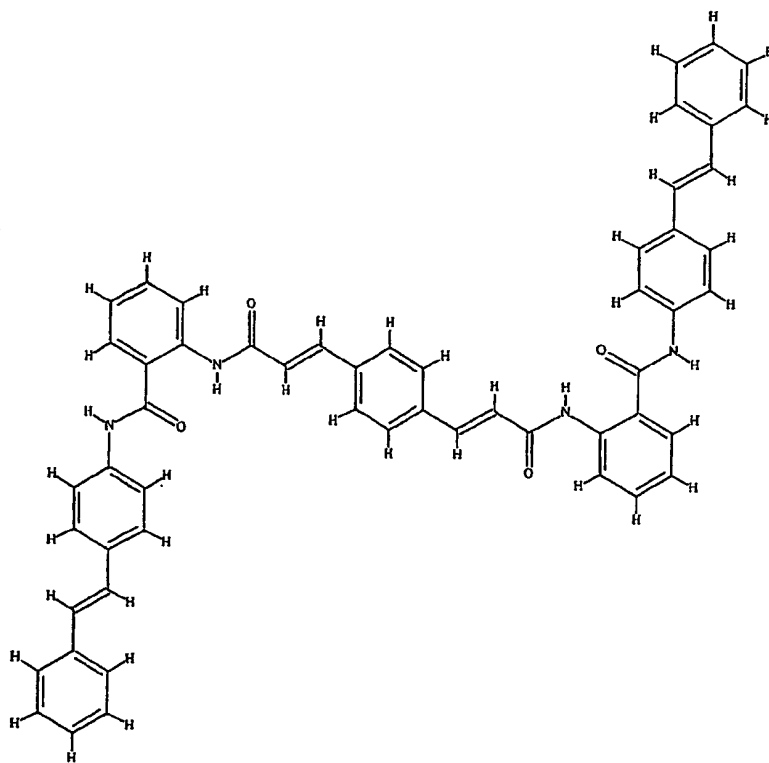
SUBSTITUTE SHEET (RULE 26)

119/248

237

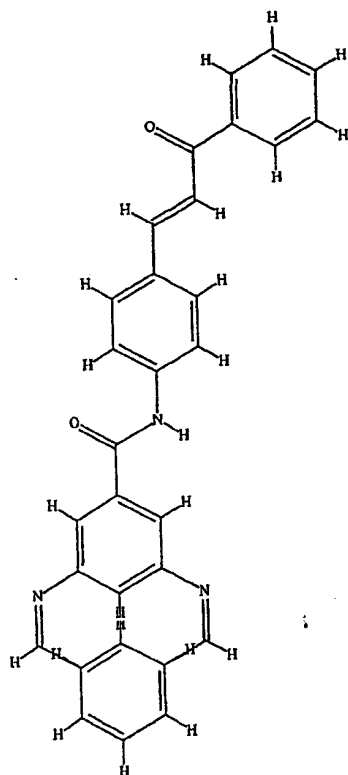


238

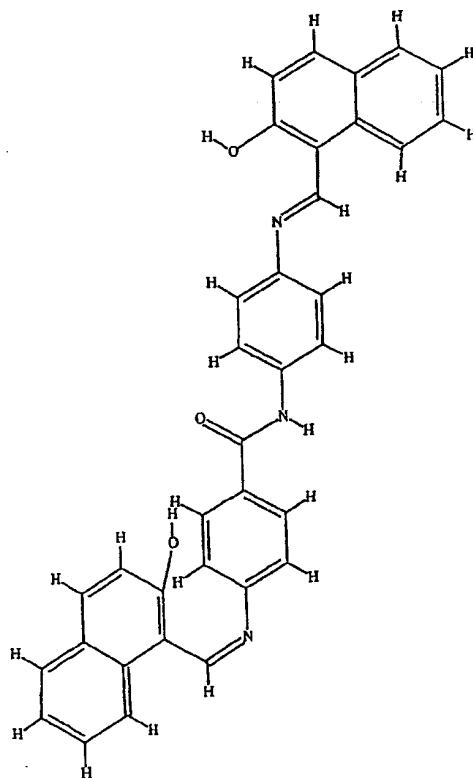


SUBSTITUTE SHEET (RULE 26)

239



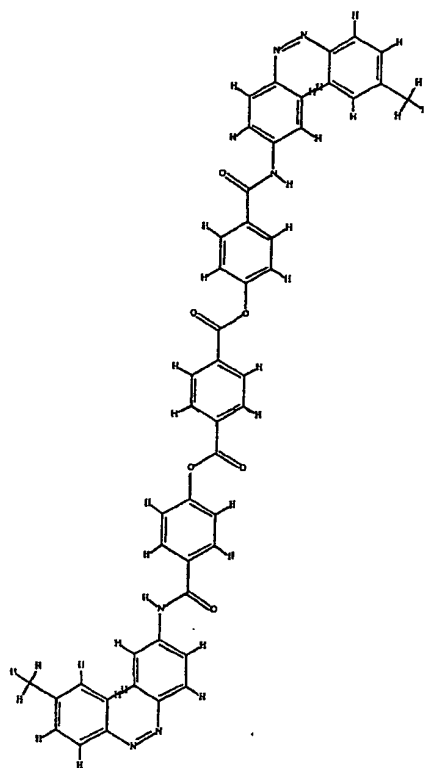
240



SUBSTITUTE SHEET (RULE 26)

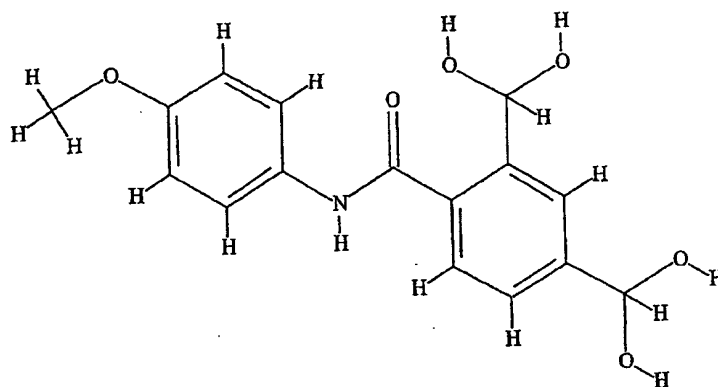
121/248

241

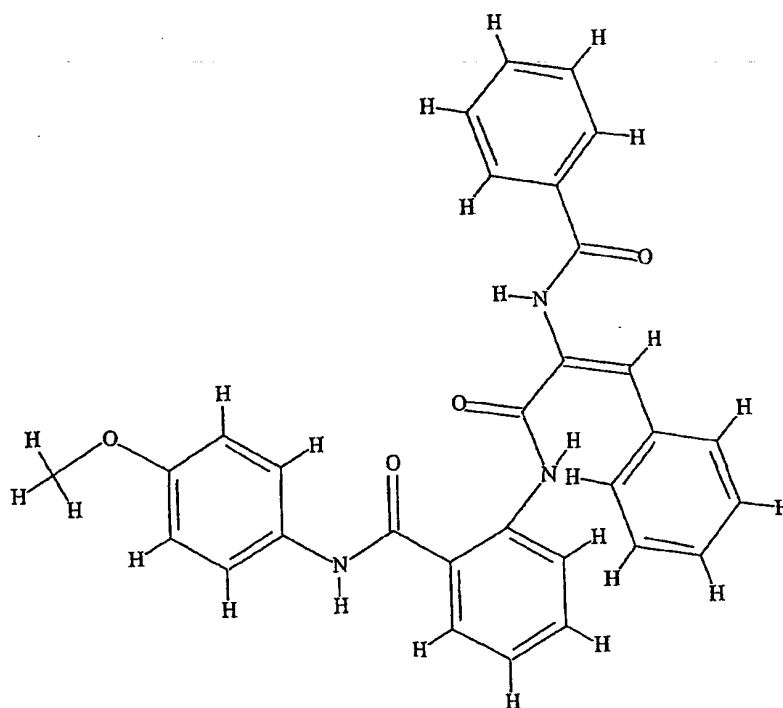


122/248

243



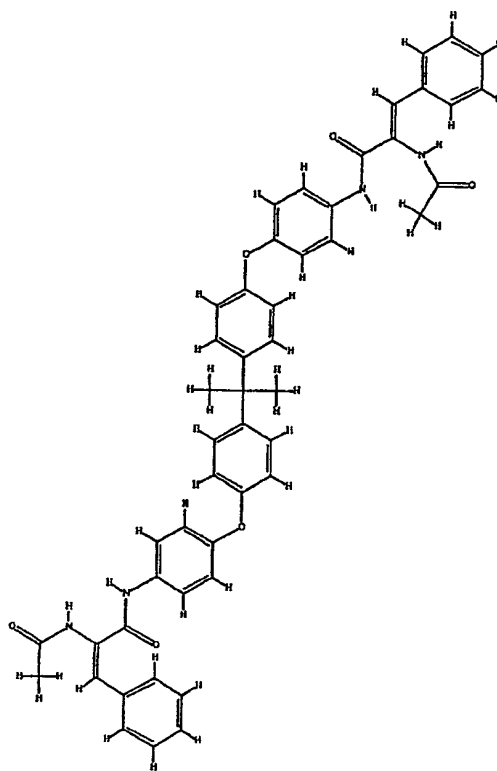
244



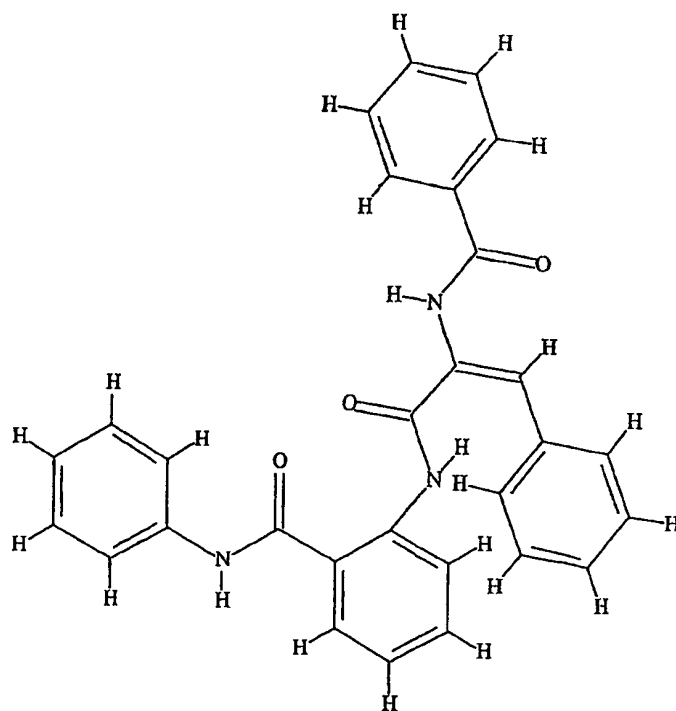
SUBSTITUTE SHEET (RULE 26)

123/248

245

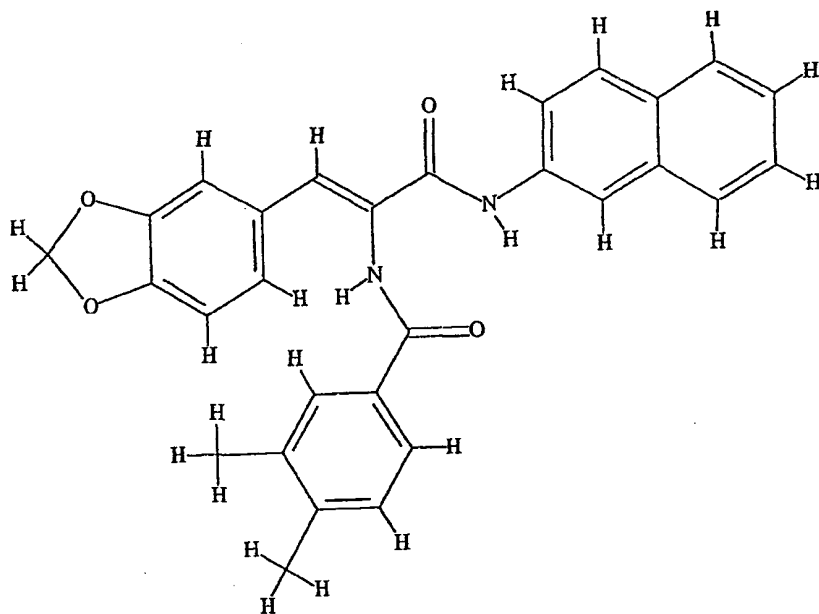


246

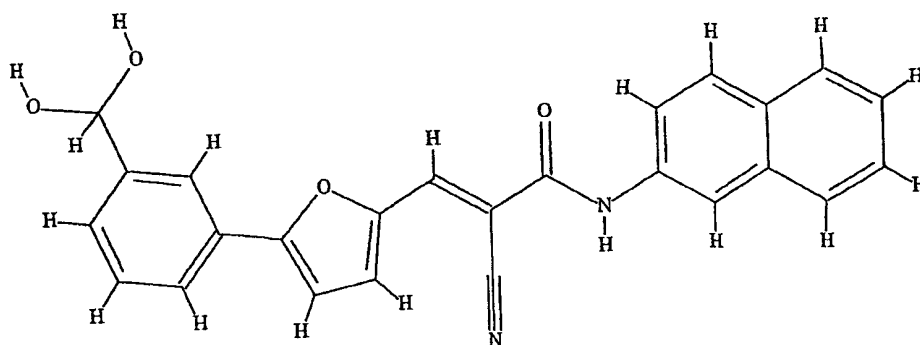


SUBSTITUTE SHEET (RULE 26)

247

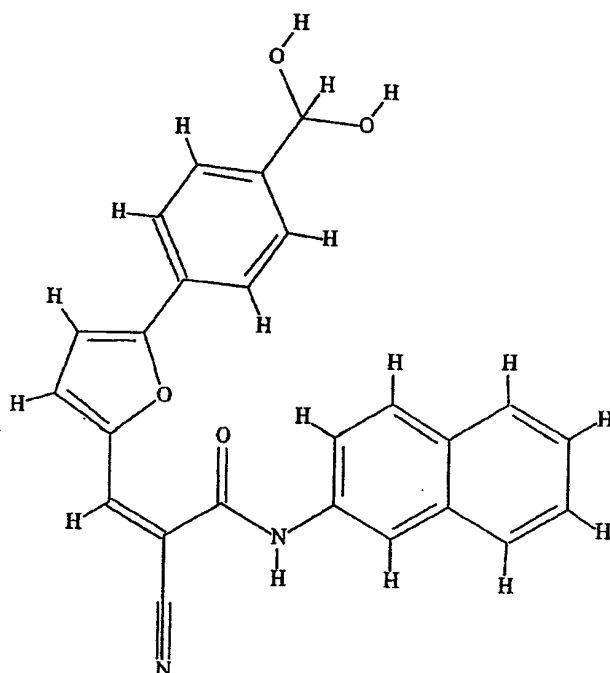


248

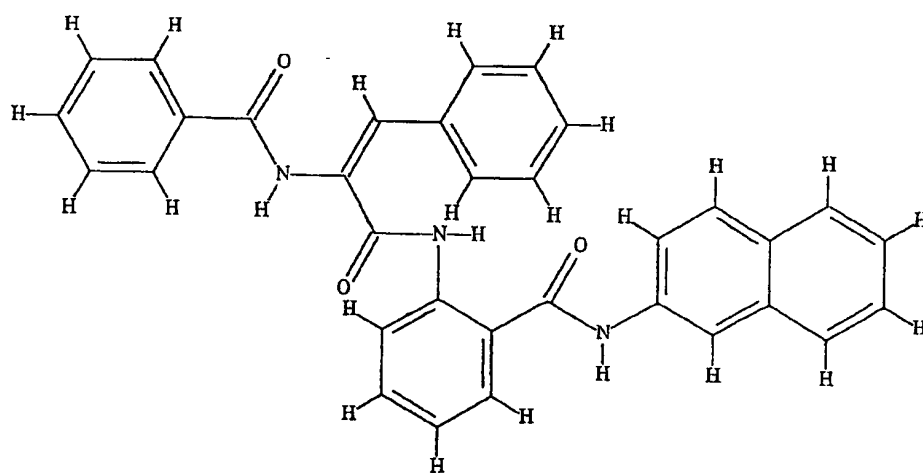


SUBSTITUTE SHEET (RULE 26)

249



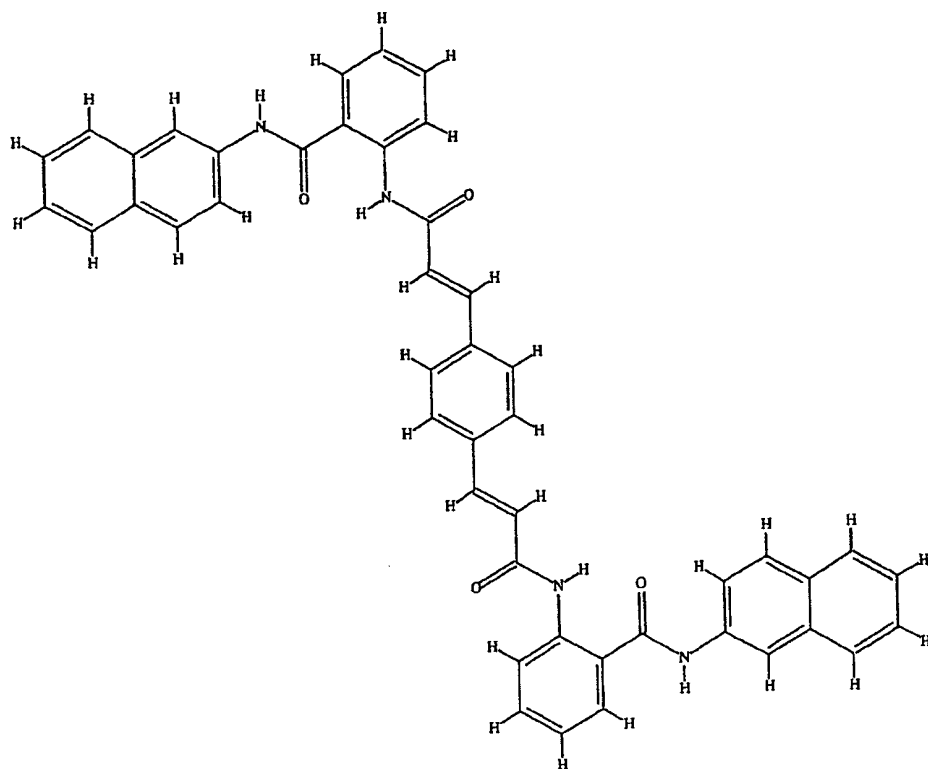
250



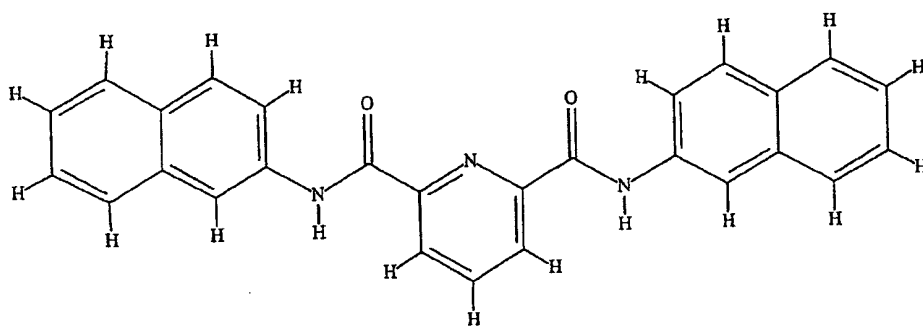


126/248

251

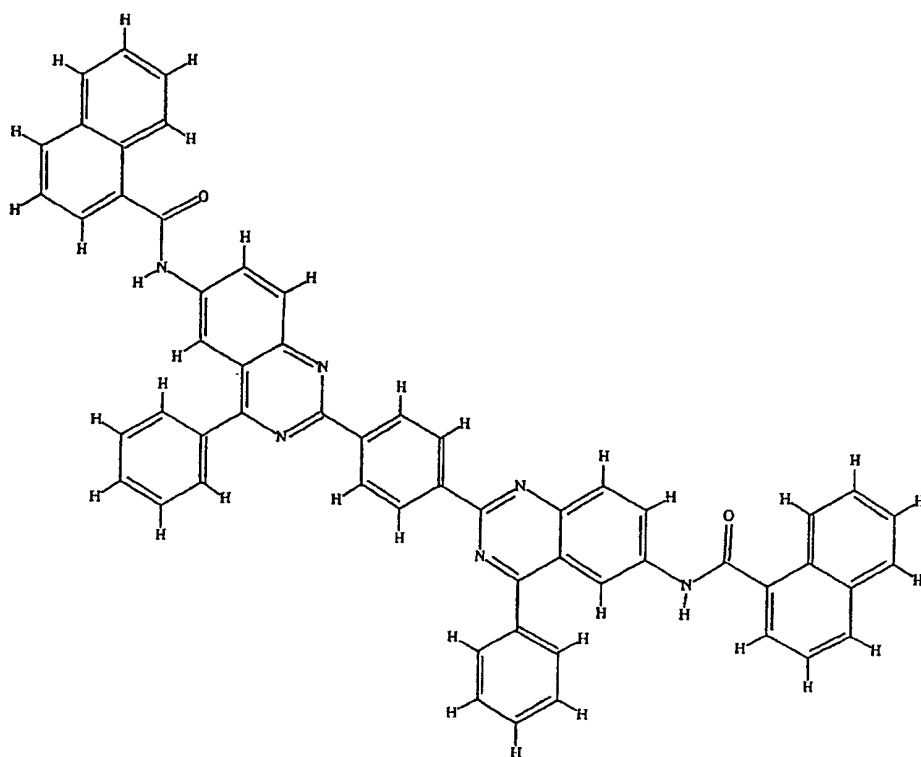


252

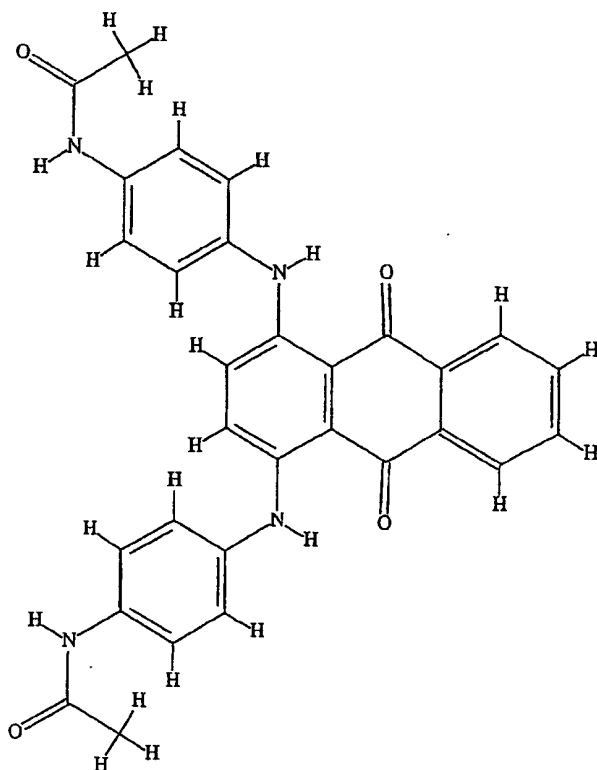


SUBSTITUTE SHEET (RULE 26)

253



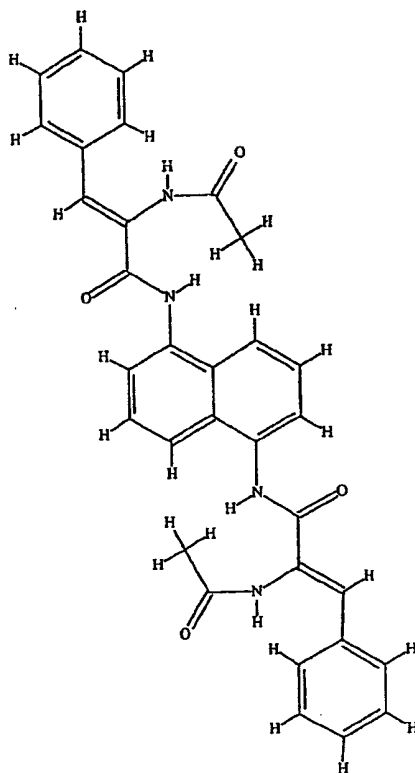
254



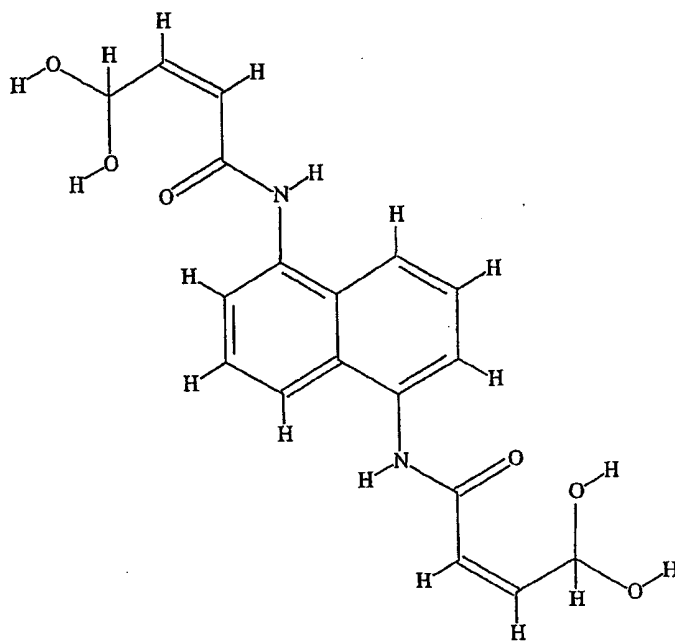
SUBSTITUTE SHEET (RULE 26)

128/248

255

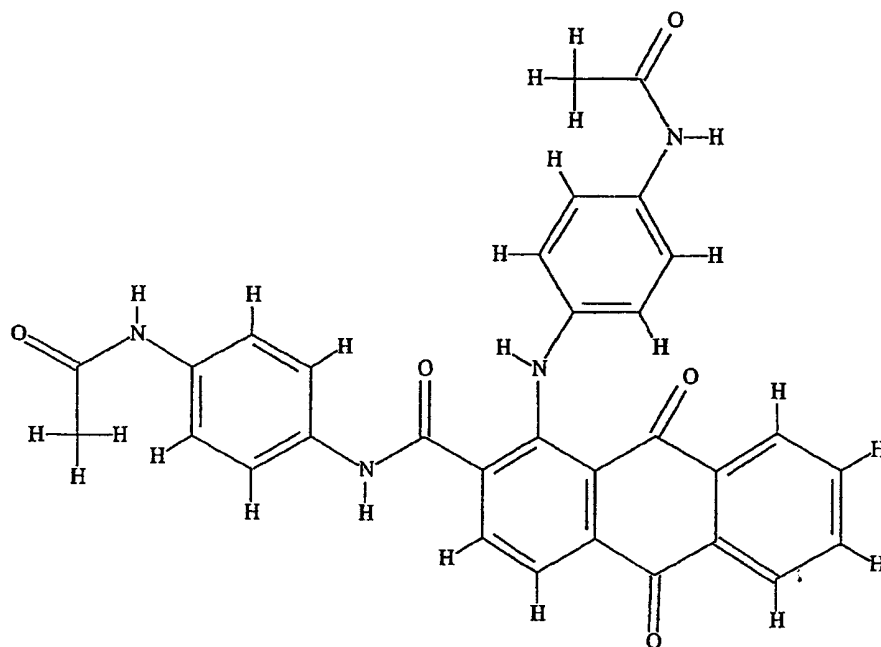


256

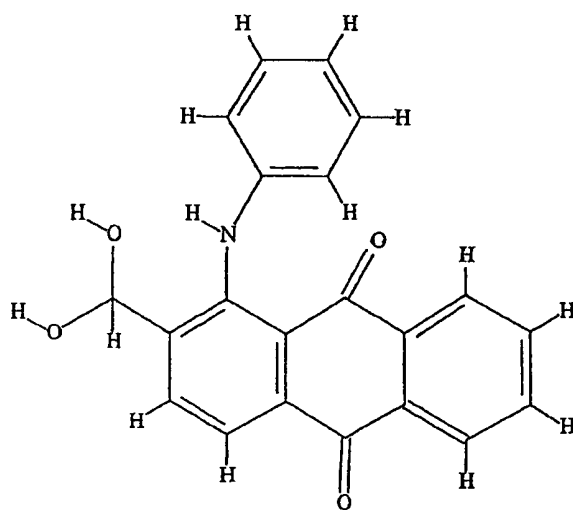


SUBSTITUTE SHEET (RULE 26)

257



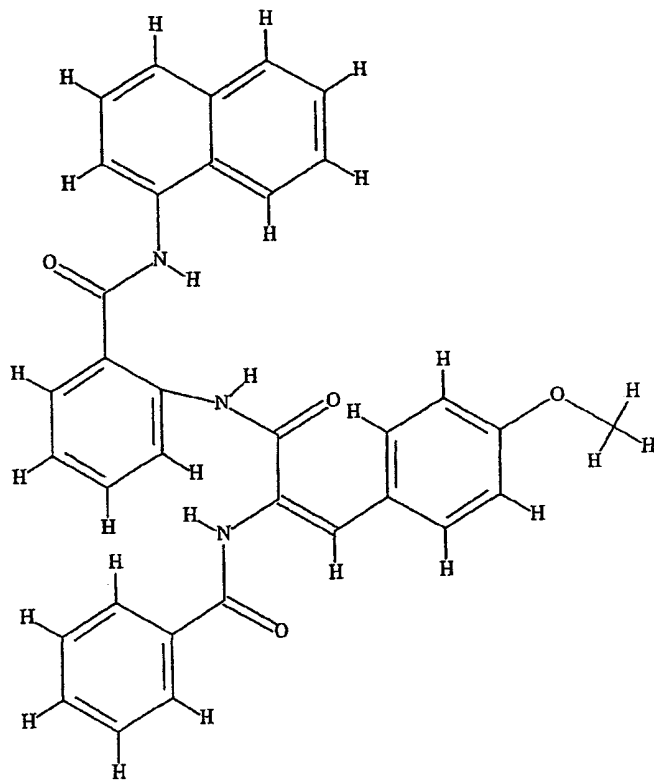
258



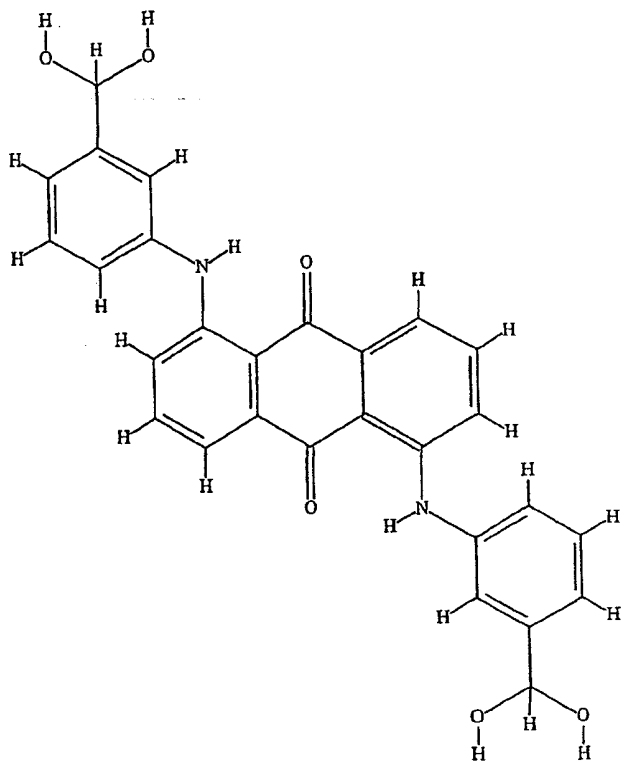
SUBSTITUTE SHEET (RULE 26)

130/248

259

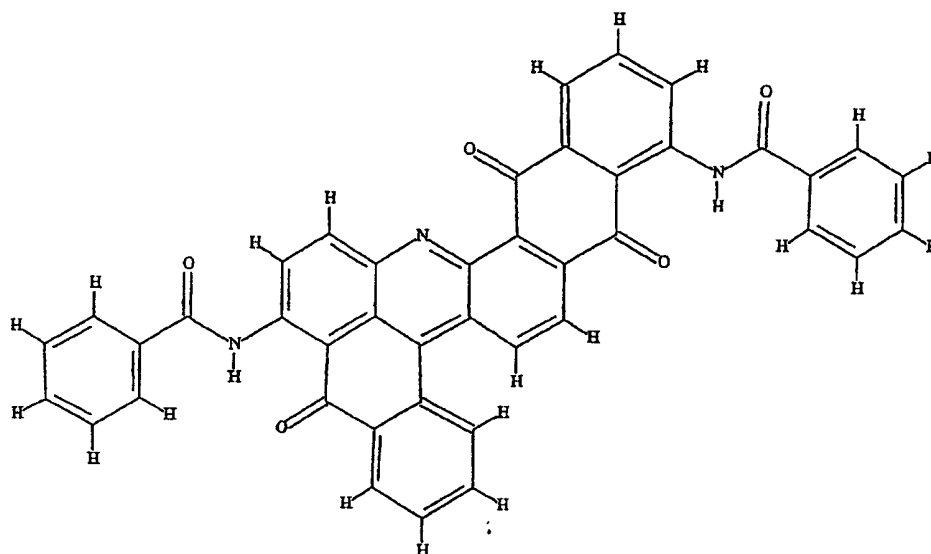


260

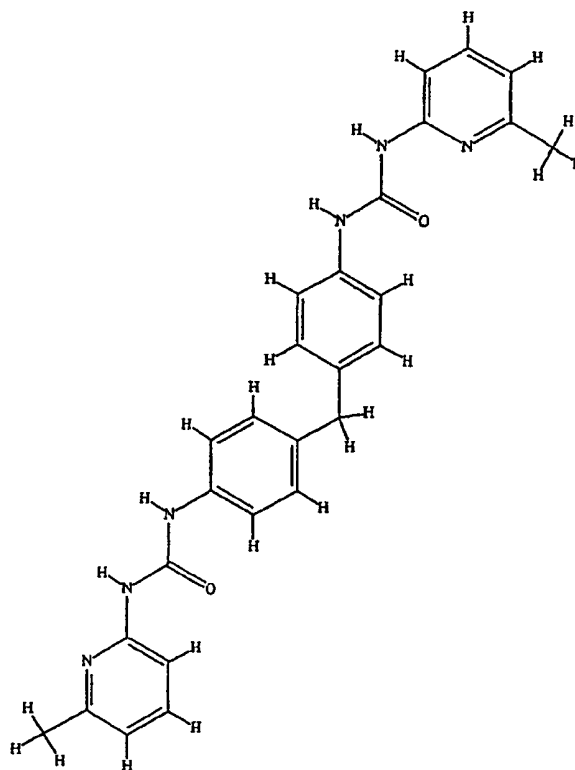


SUBSTITUTE SHEET (RULE 26)

261

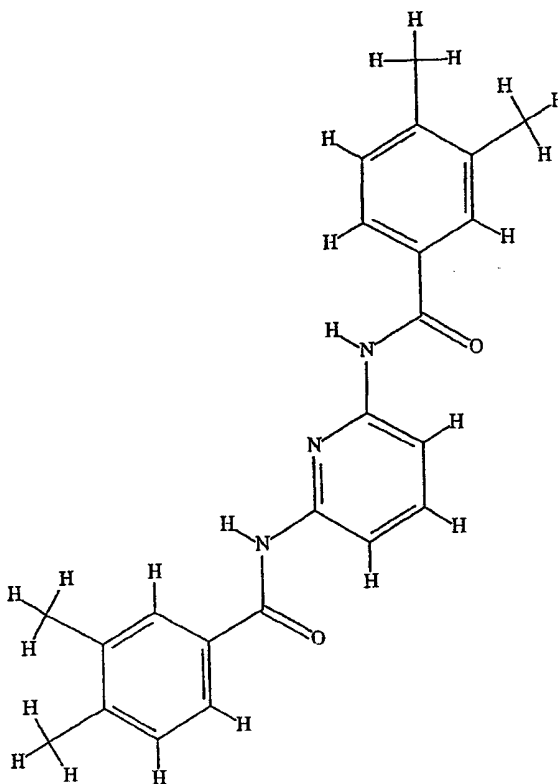


262

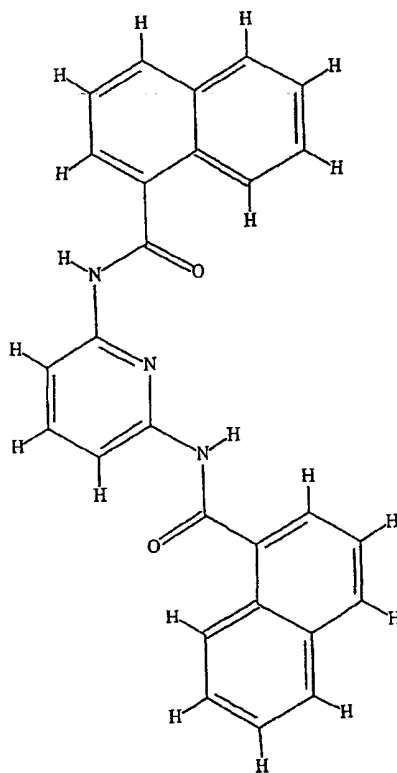


SUBSTITUTE SHEET (RULE 26)

263

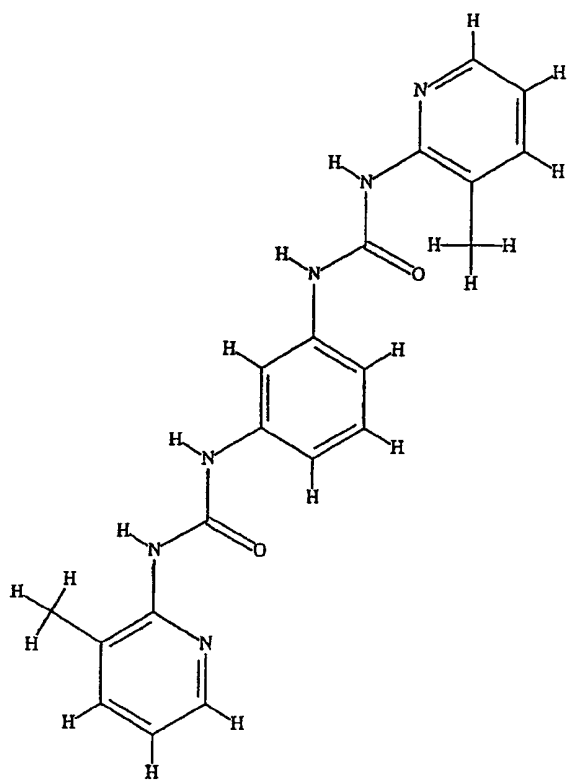


264

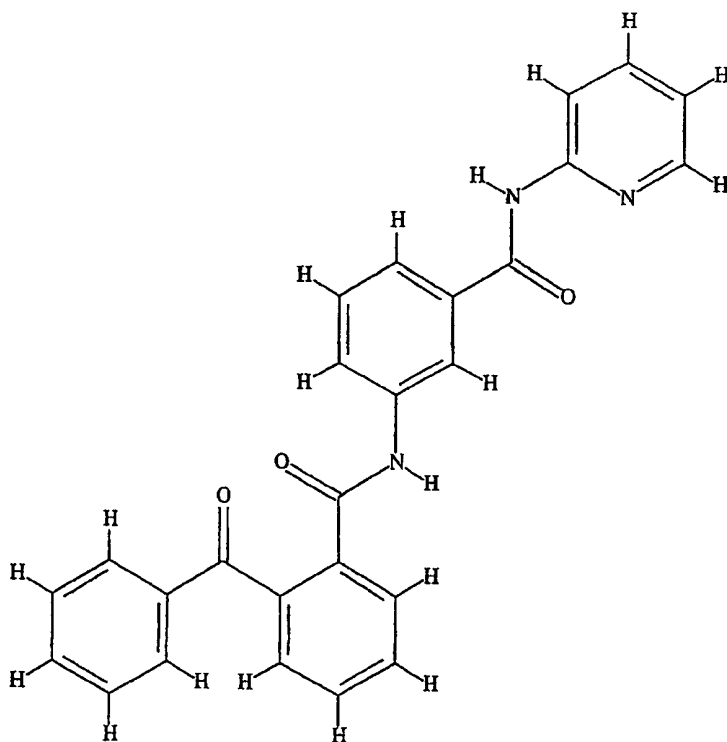


SUBSTITUTE SHEET (RULE 26)

265



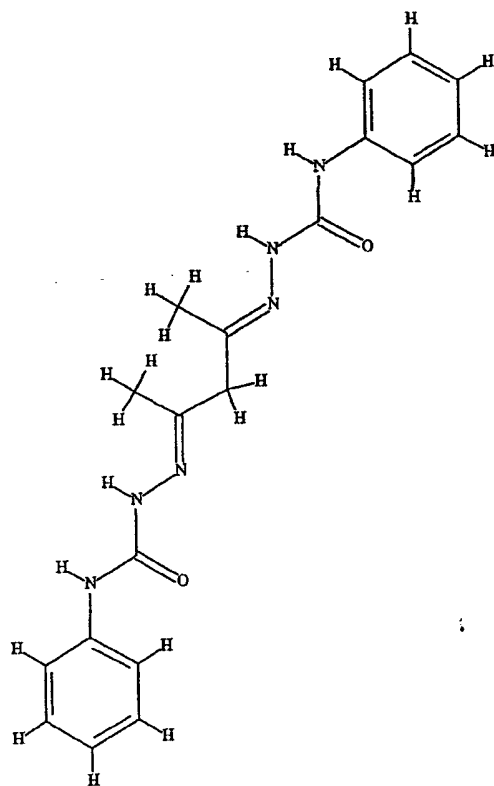
266



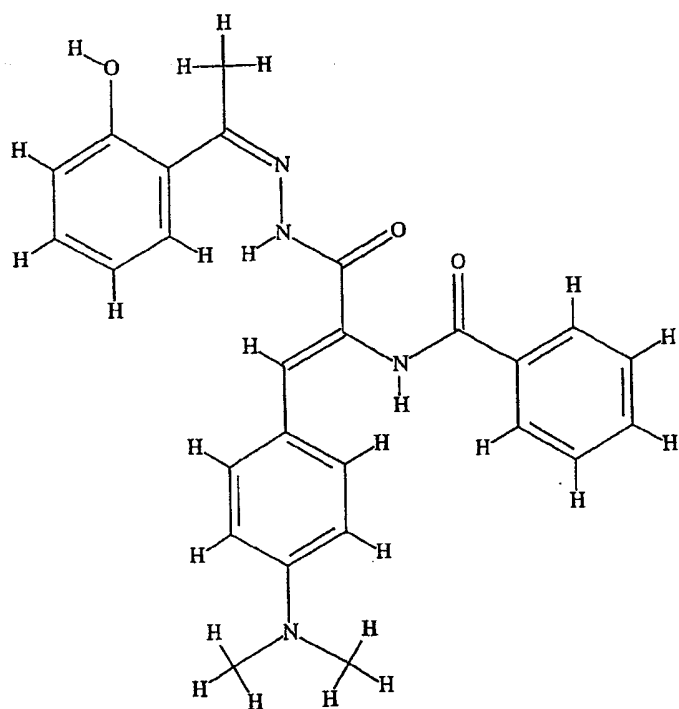


134/248

267

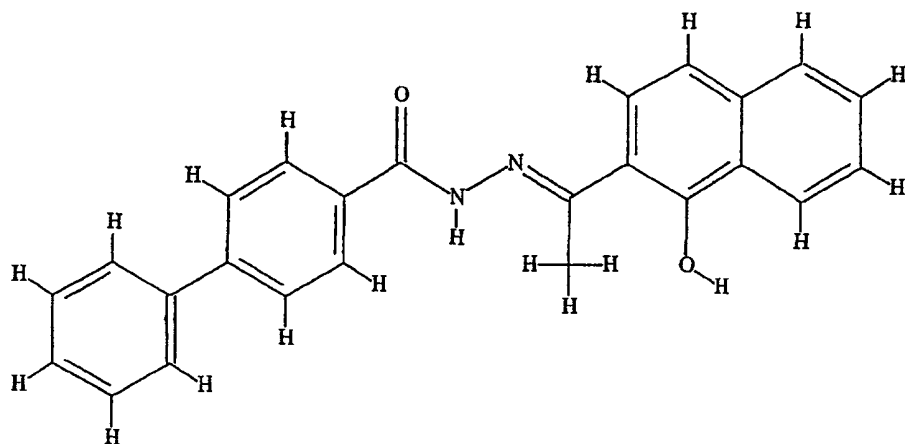


268

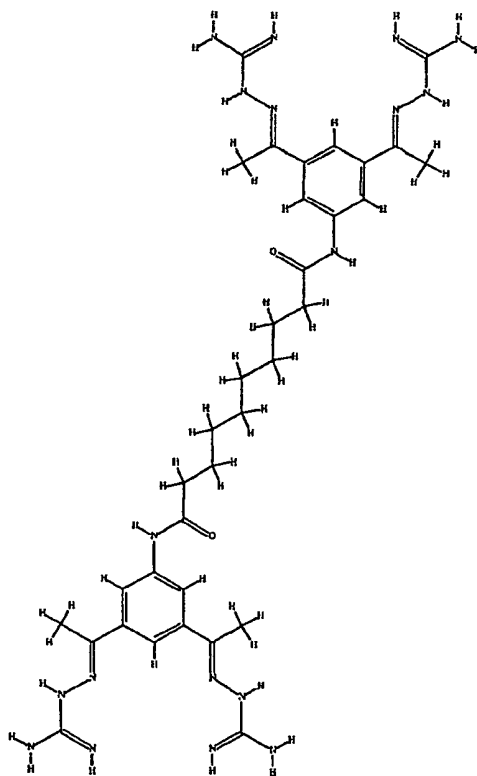


SUBSTITUTE SHEET (RULE 26)

269



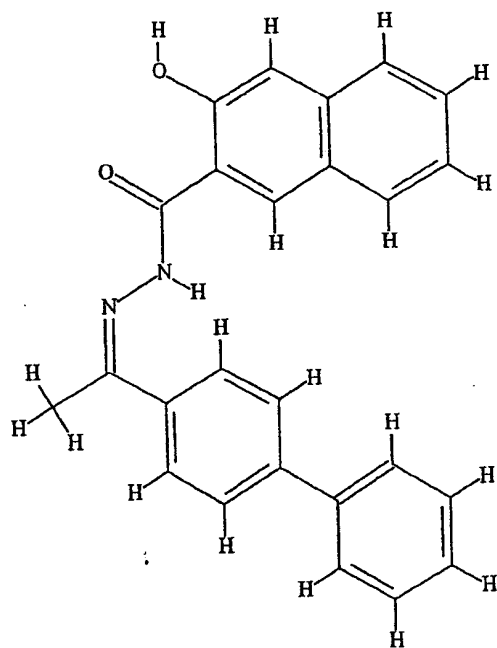
270



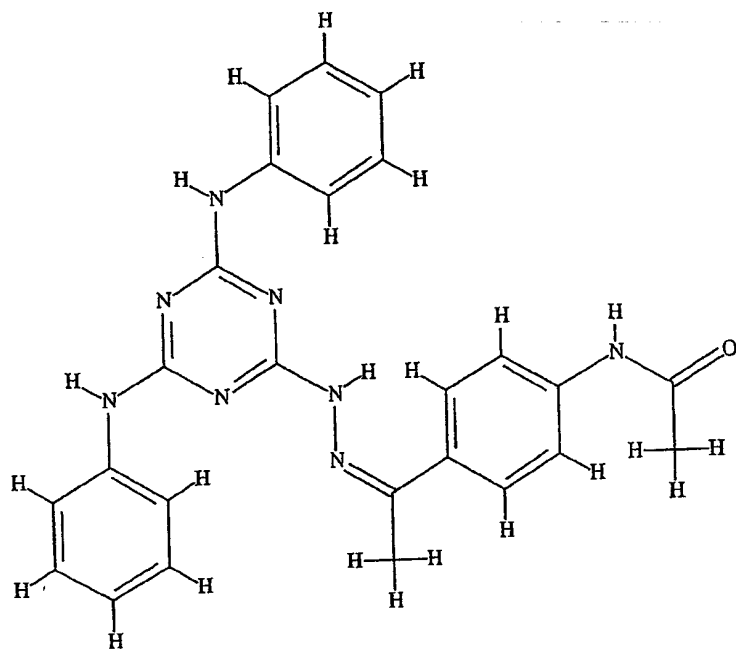
SUBSTITUTE SHEET (RULE 26)

136/248

271



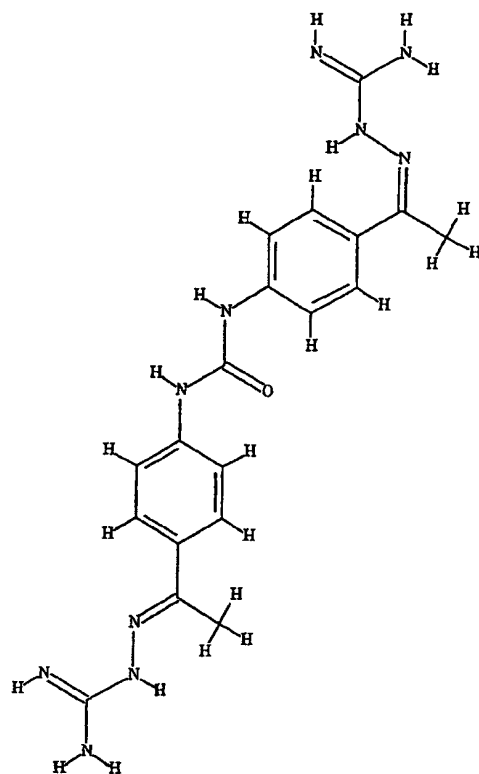
272



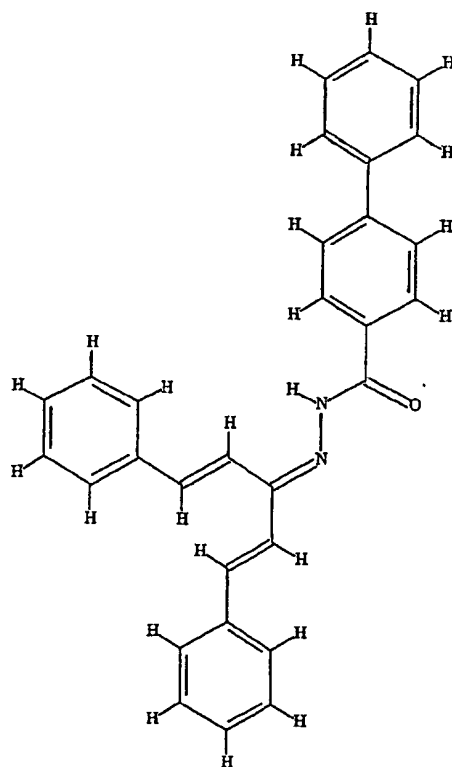
SUBSTITUTE SHEET (RULE 26)

137/248

273

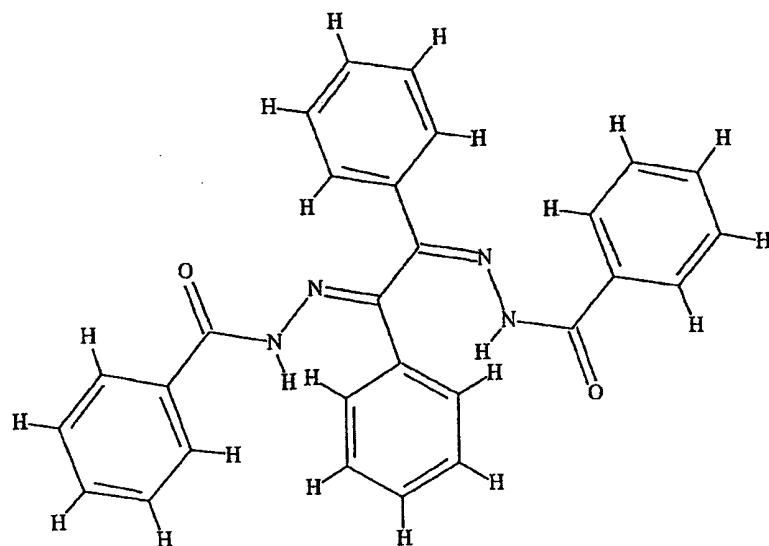


274

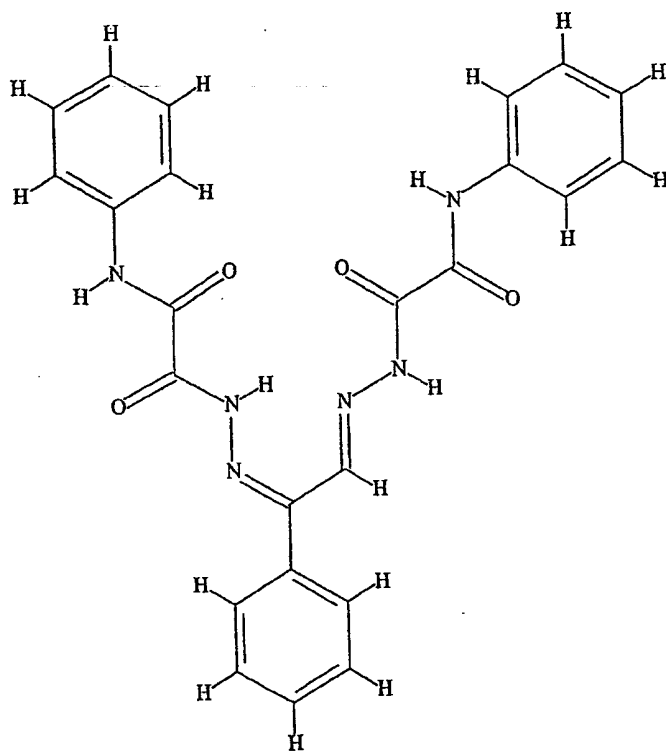


SUBSTITUTE SHEET (RULE 26)

275



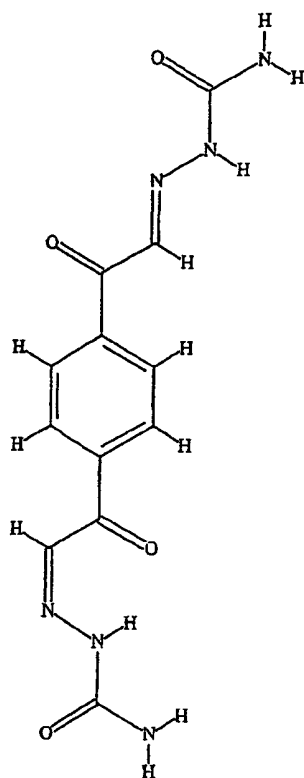
276



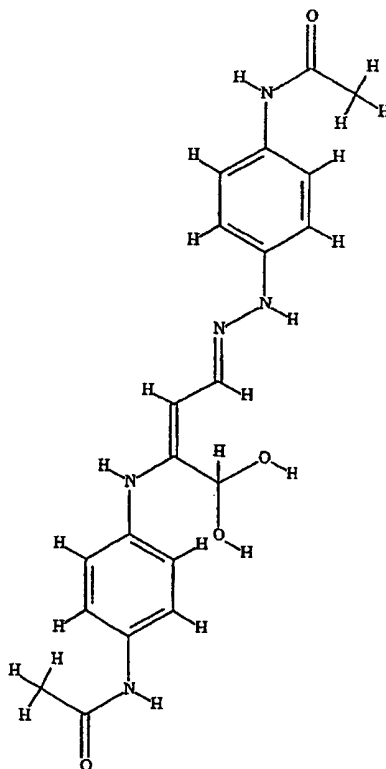
SUBSTITUTE SHEET (RULE 26)

139/248

277

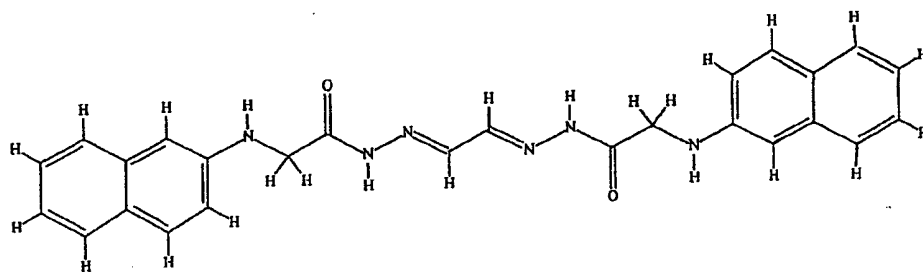


278

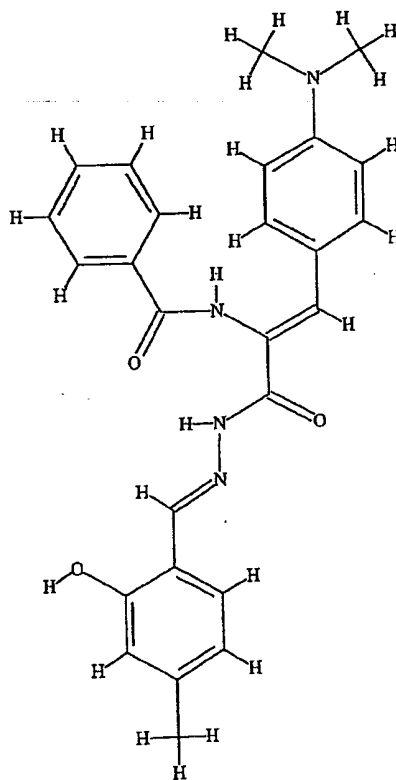


SUBSTITUTE SHEET (RULE 26)

279

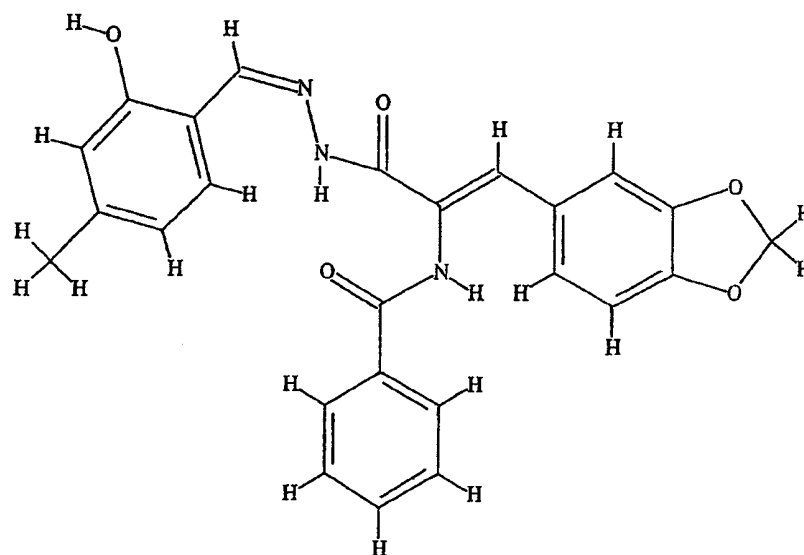


280

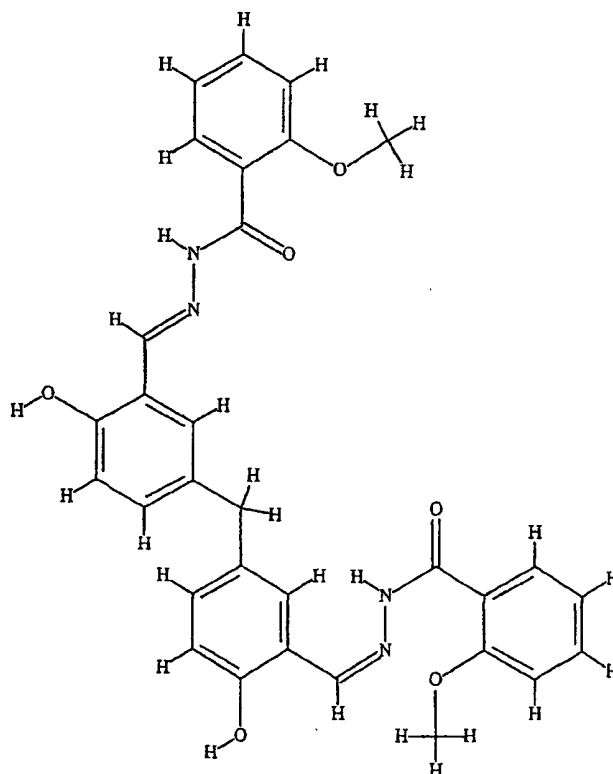


SUBSTITUTE SHEET (RULE 26)

281



282

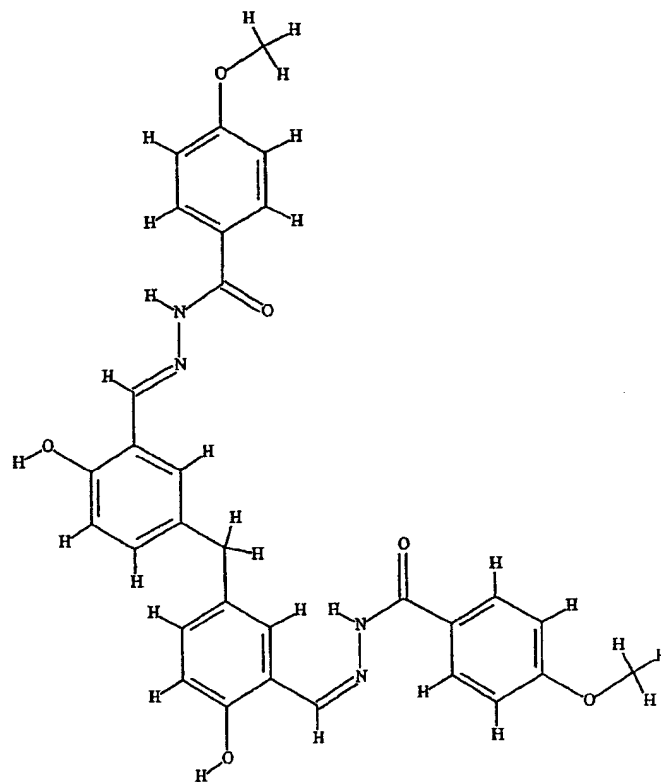


SUBSTITUTE SHEET (RULE 26)

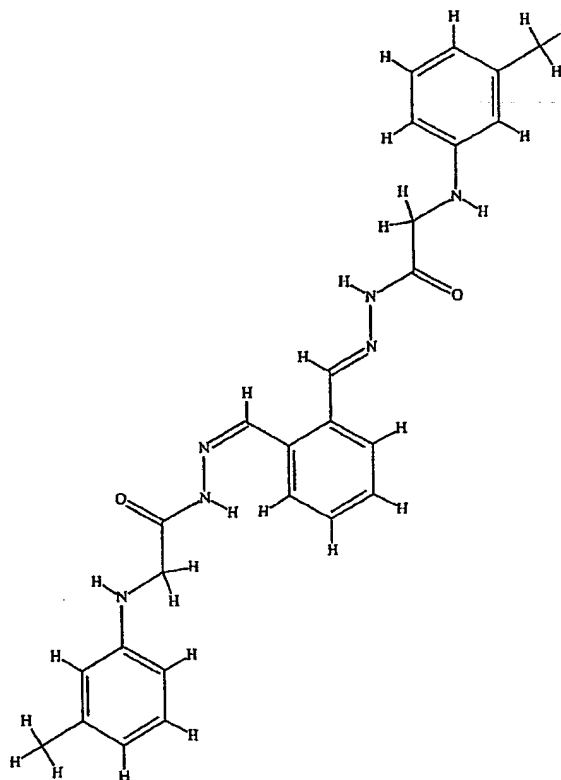


142/248

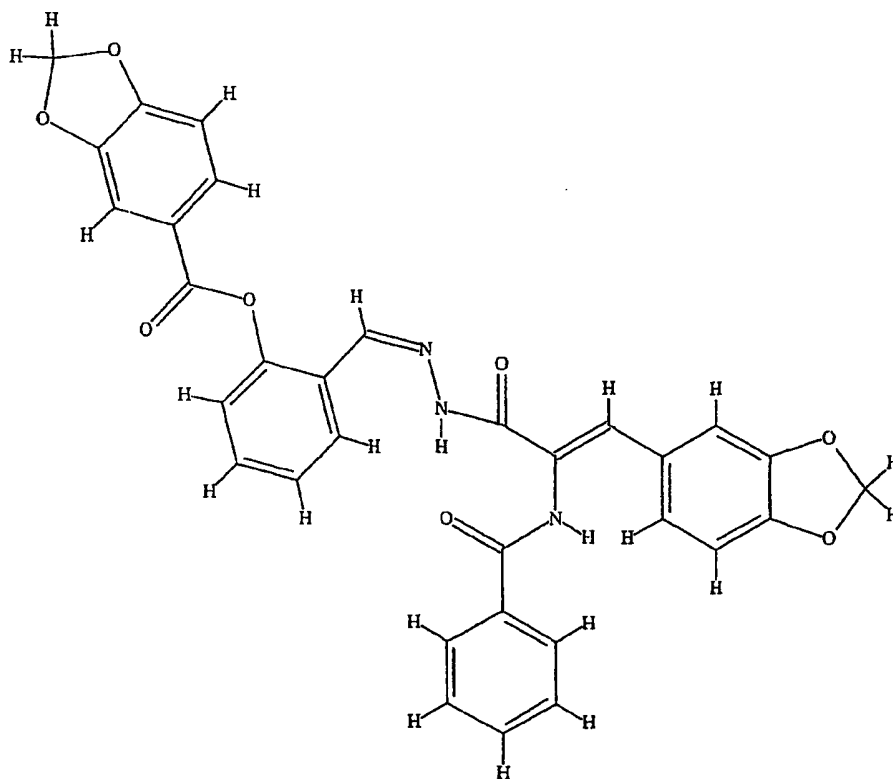
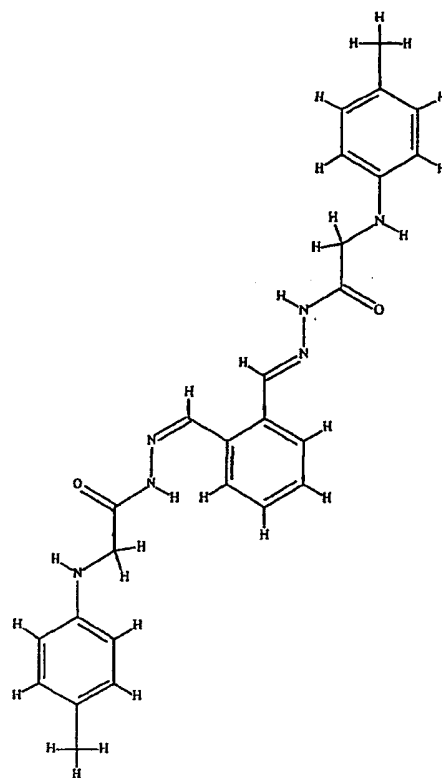
283



284



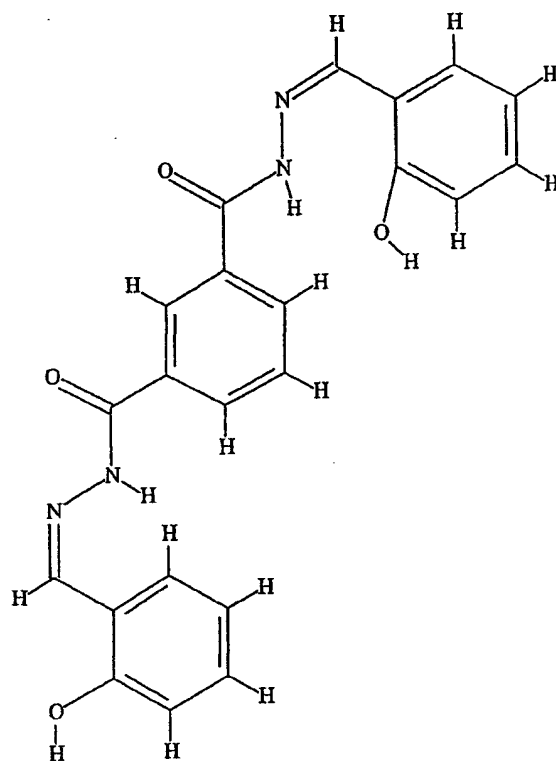
SUBSTITUTE SHEET (RULE 26)



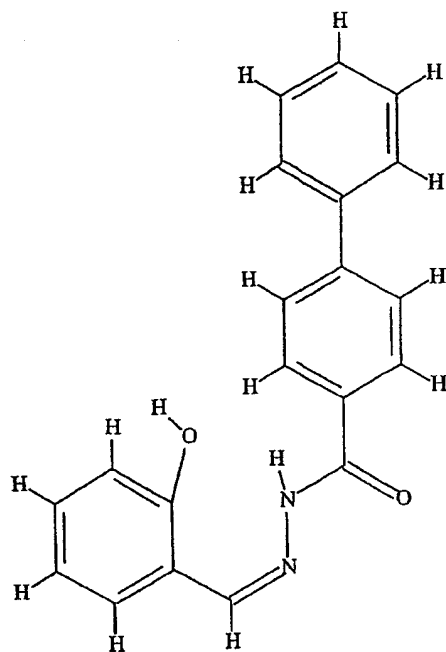
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144/248

287

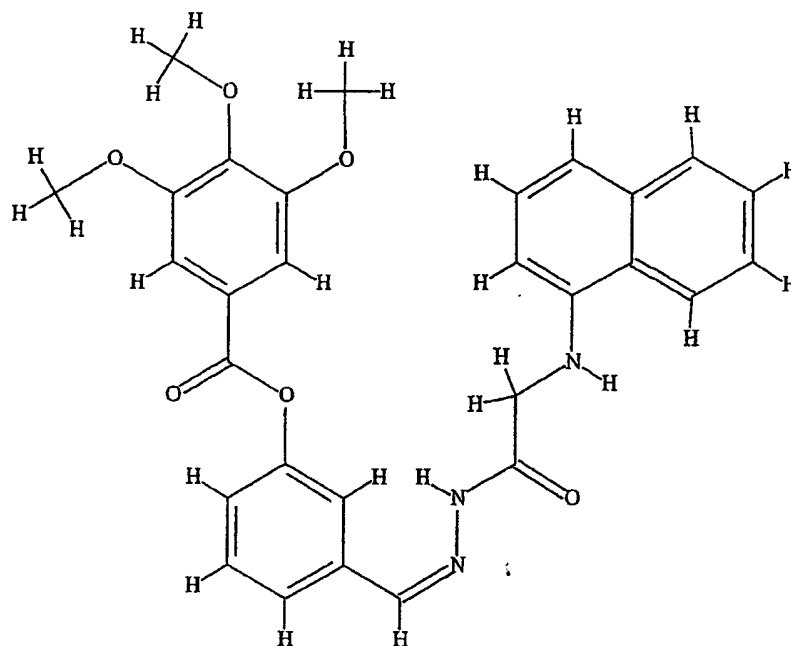


288

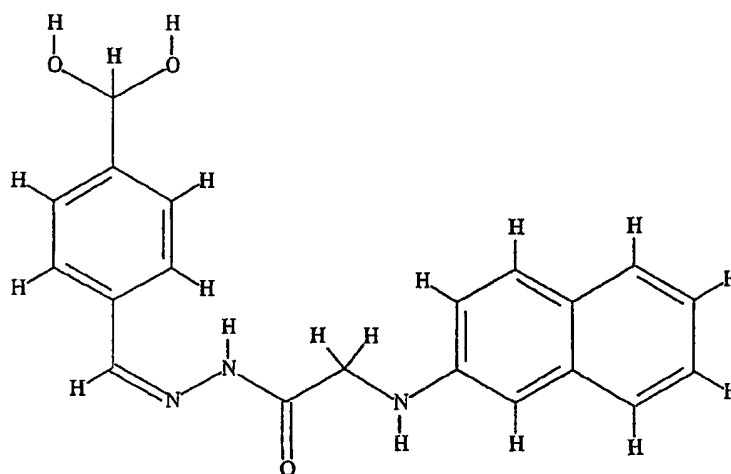


SUBSTITUTE SHEET (RULE 26)

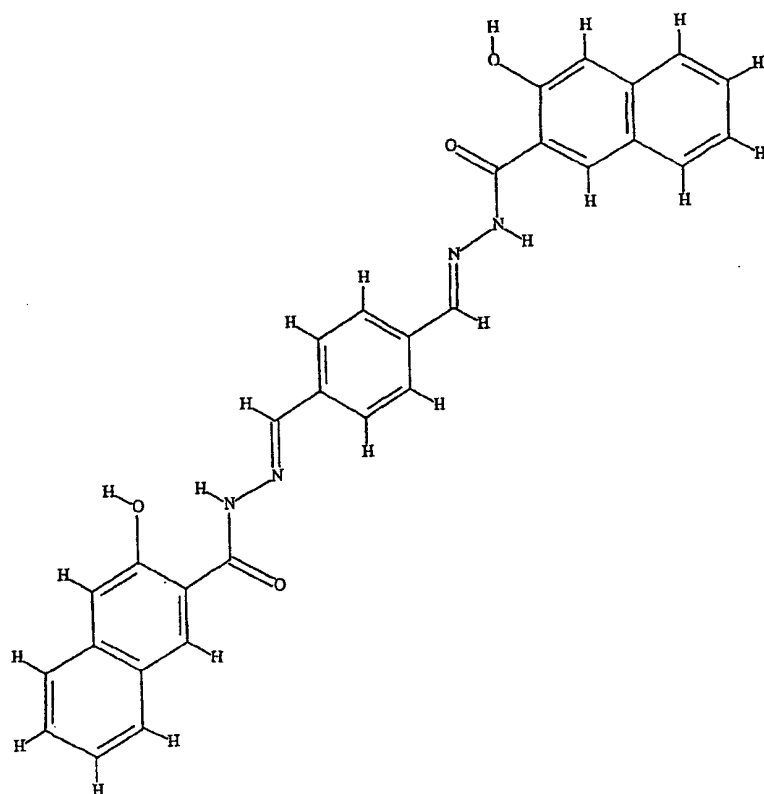
289



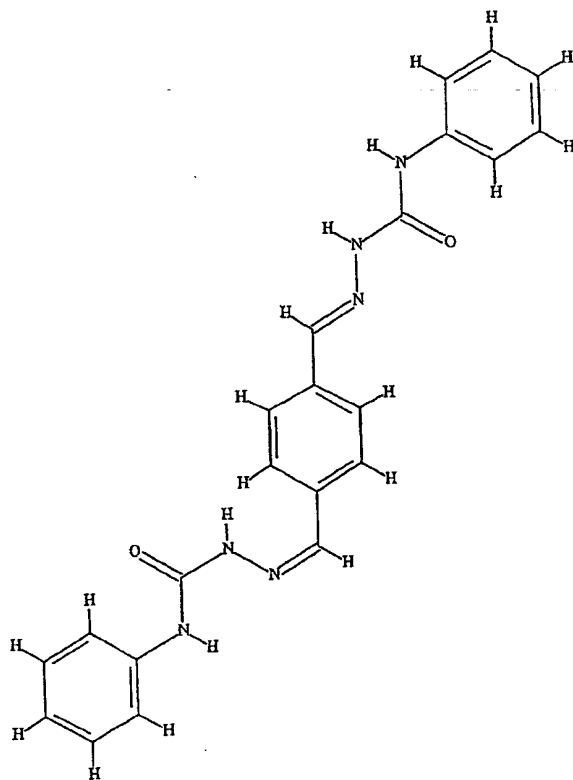
290



291



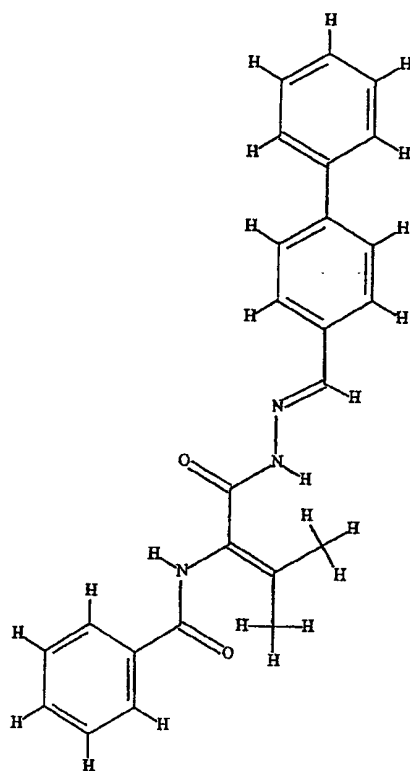
292



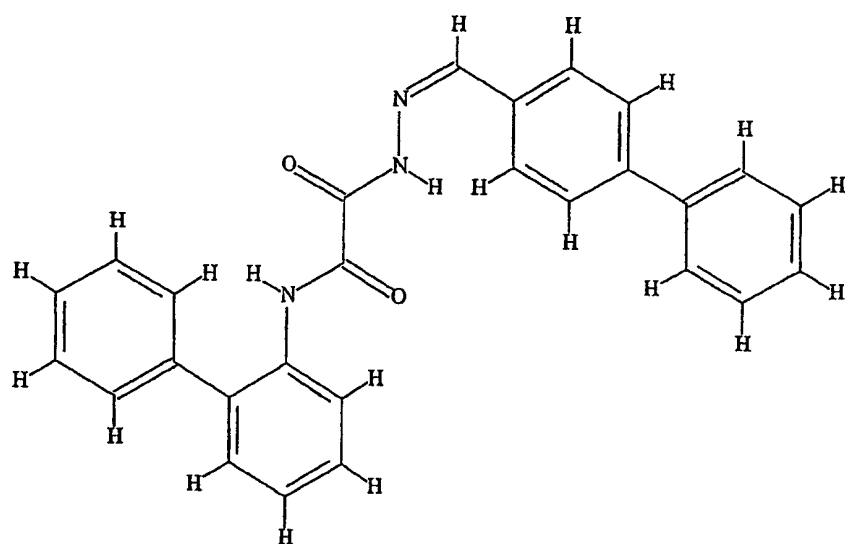
SUBSTITUTE SHEET (RULE 26)

147/248

293

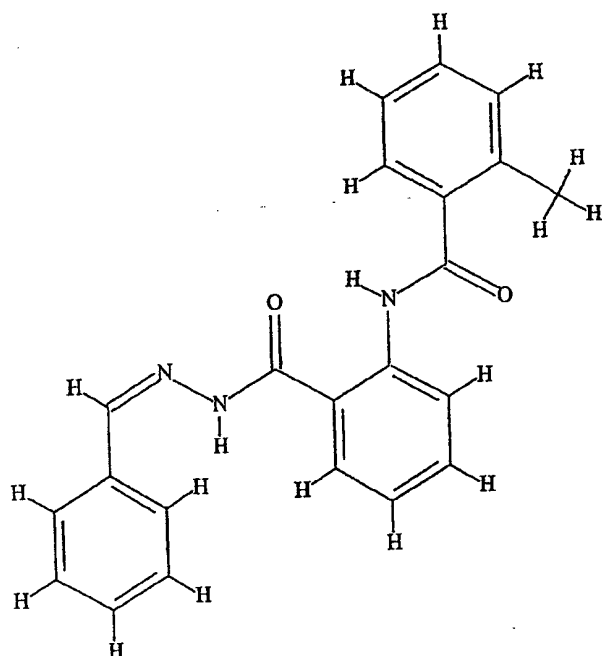


294

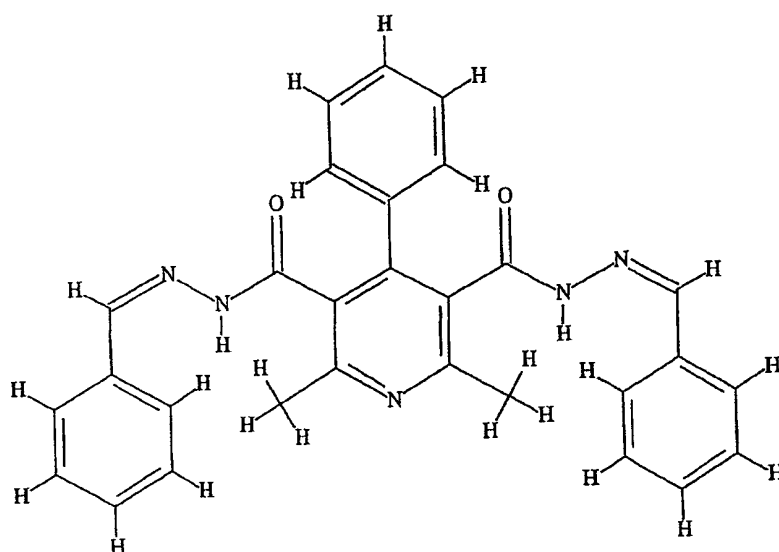


SUBSTITUTE SHEET (RULE 26)

295

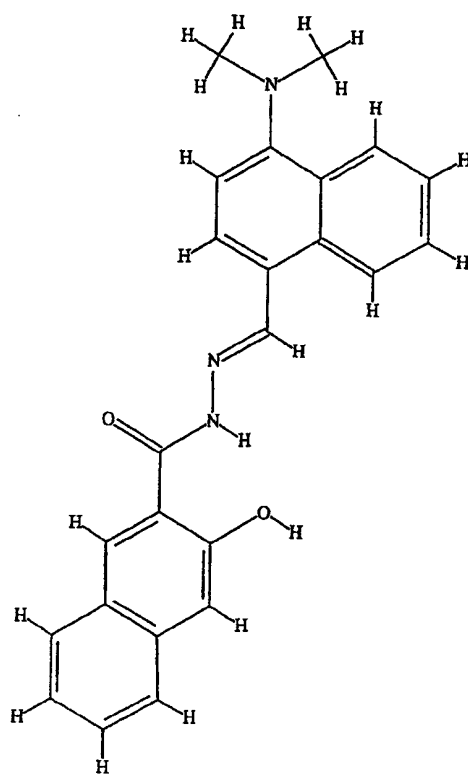


296

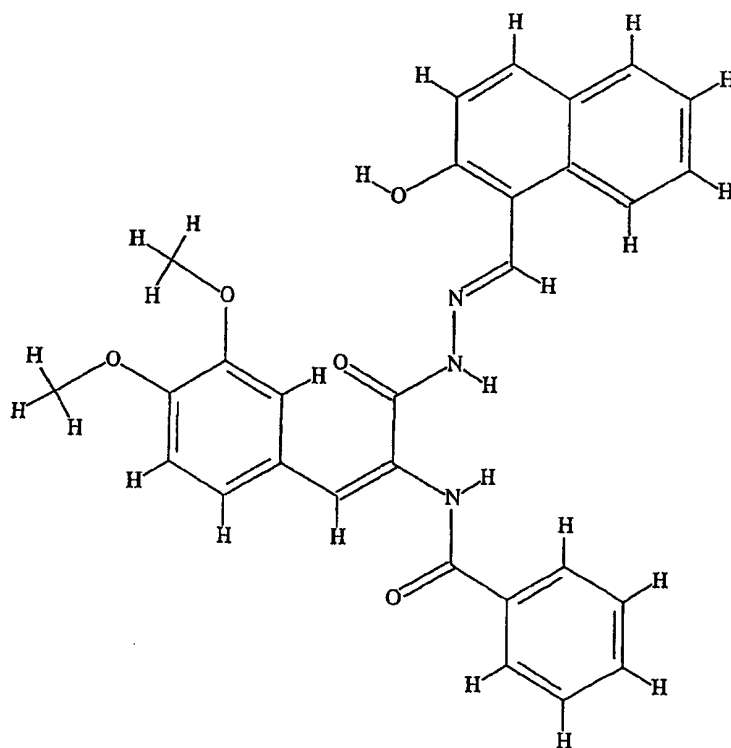


149/248

297



298

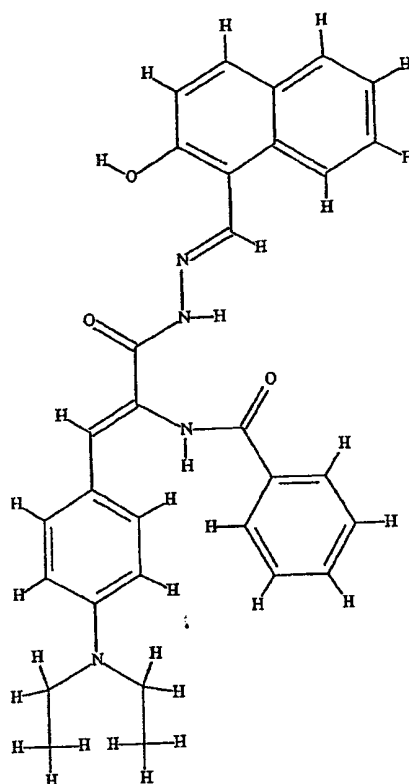


SUBSTITUTE SHEET (RULE 26)

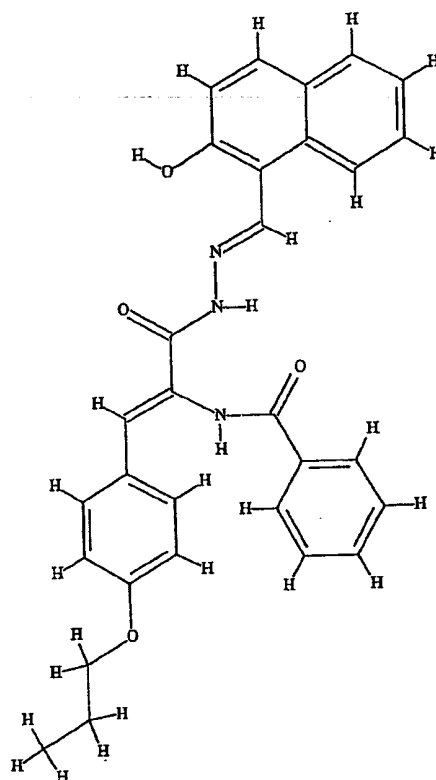


150/248

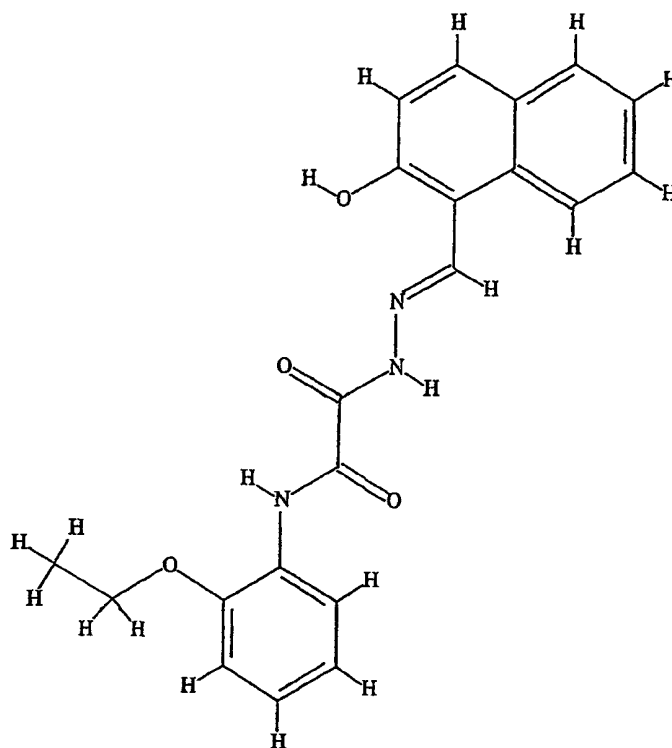
299



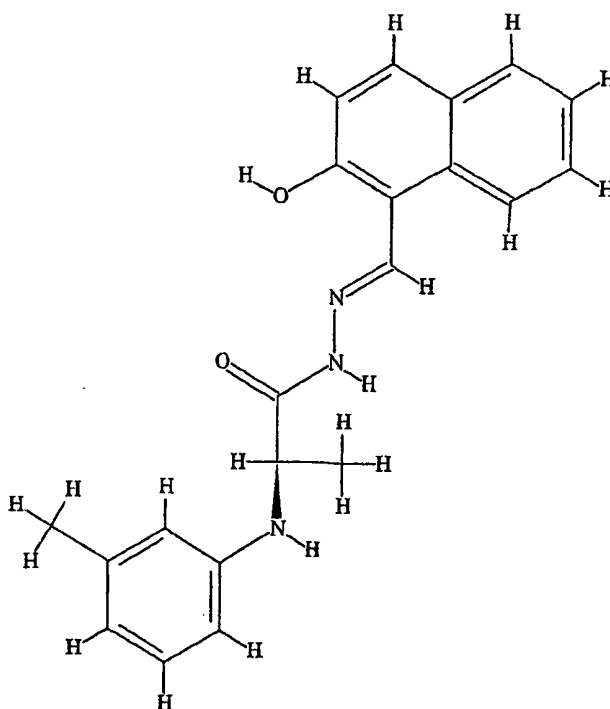
300



SUBSTITUTE SHEET (RULE 26)

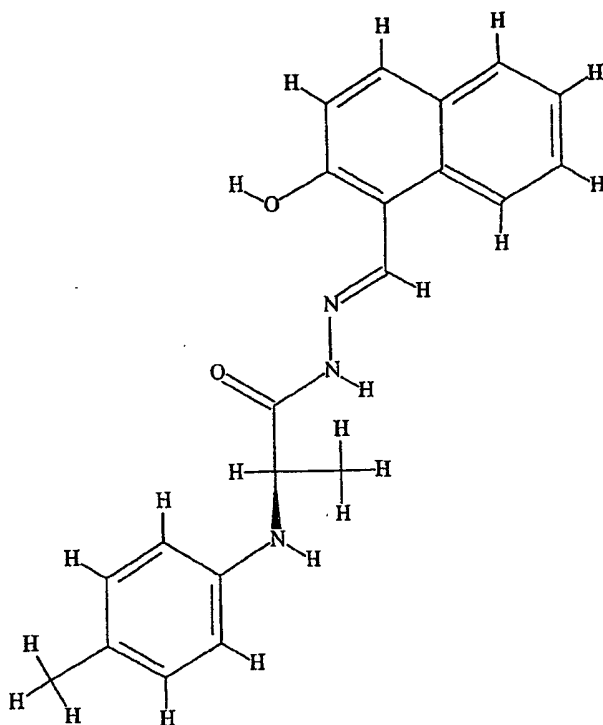


302

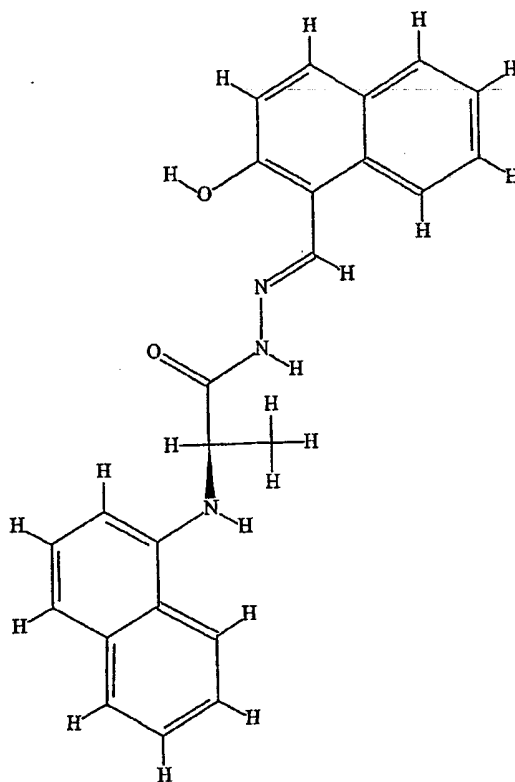


**SUBSTITUTE SHEET (RULE 26)**

303



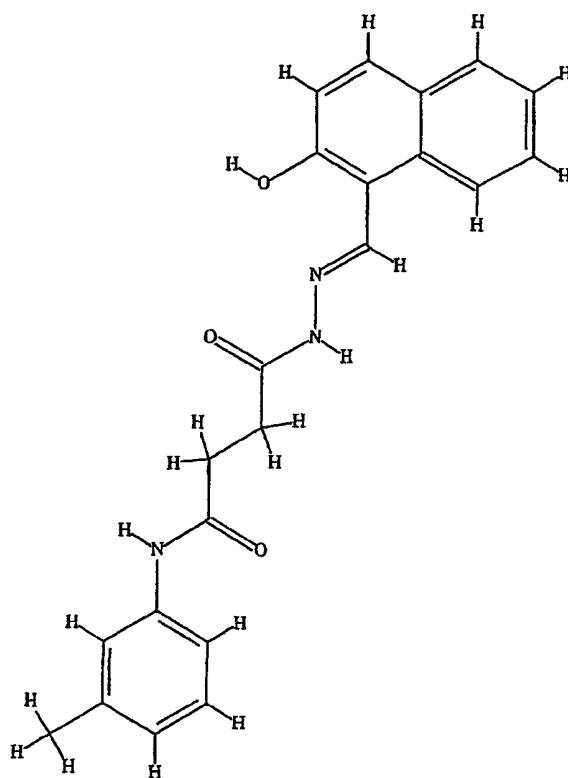
304



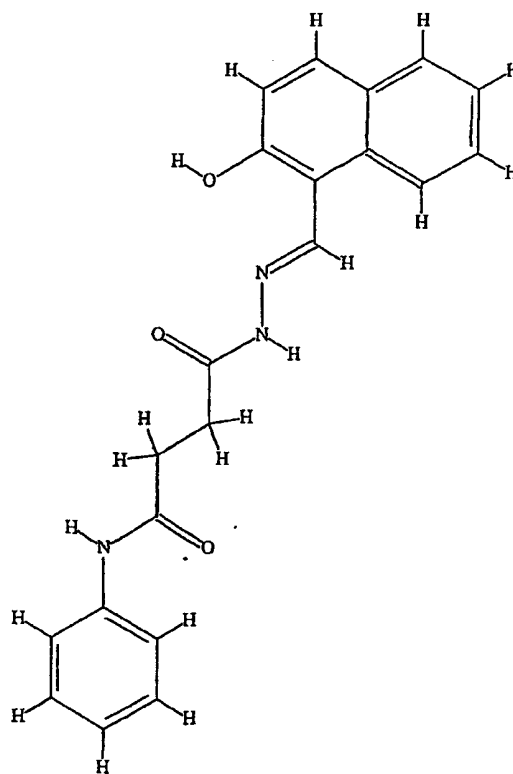
SUBSTITUTE SHEET (RULE 26)

153/248

305



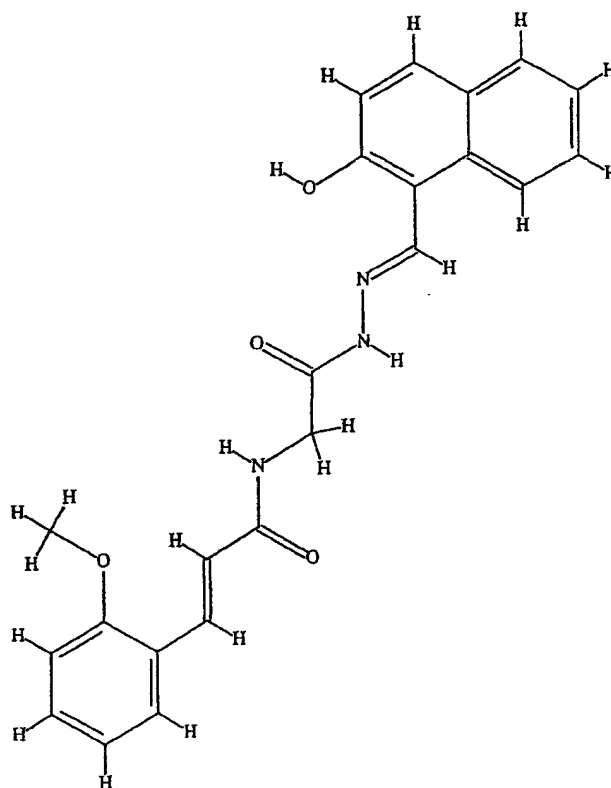
306



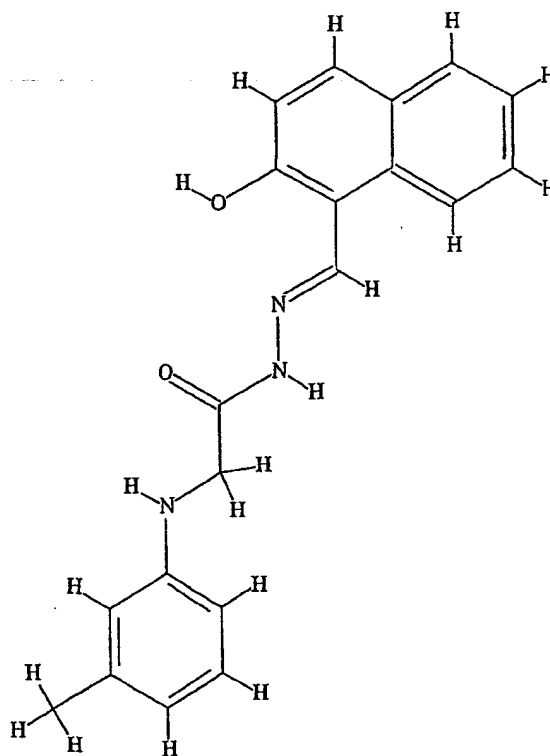
SUBSTITUTE SHEET (RULE 26)

154/248

307



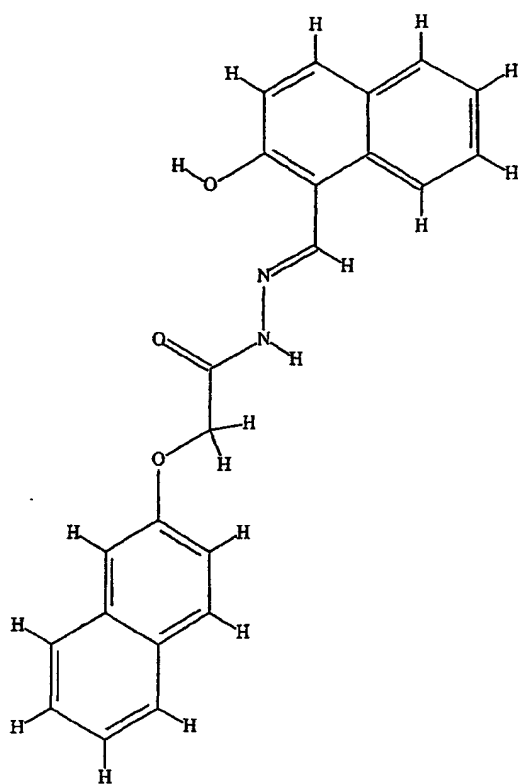
308



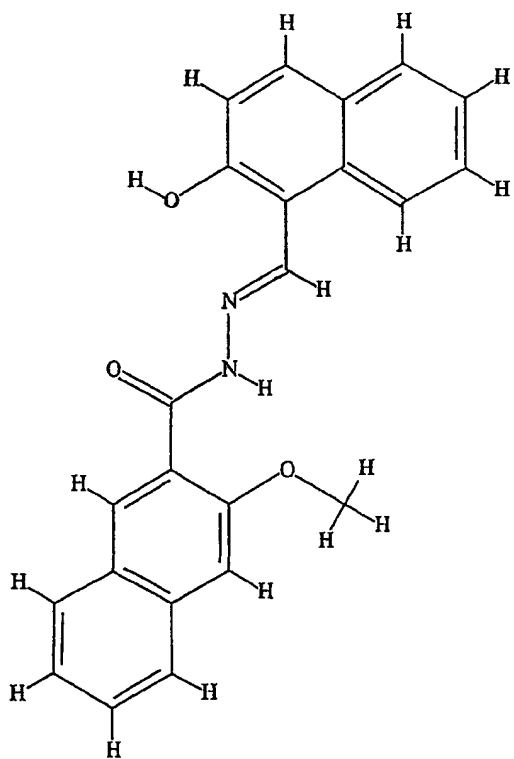
SUBSTITUTE SHEET (RULE 26)

155/248

309



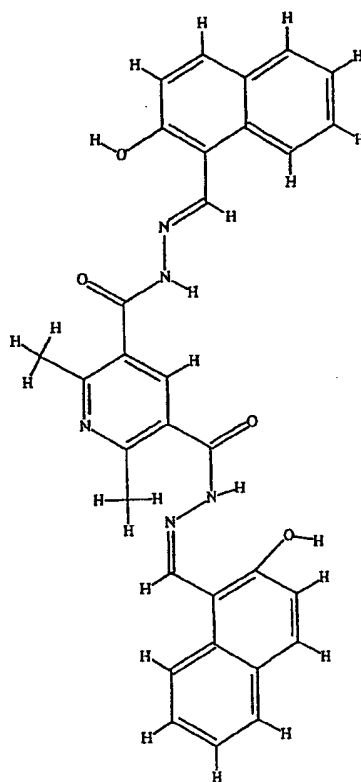
310



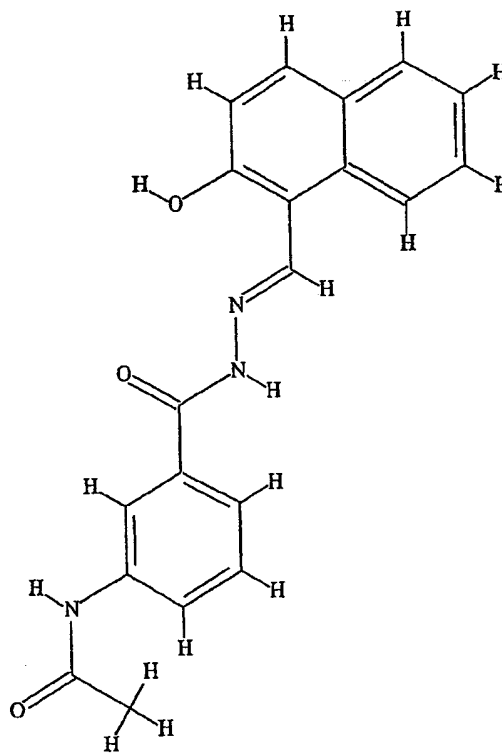
SUBSTITUTE SHEET (RULE 26)

156/248

311



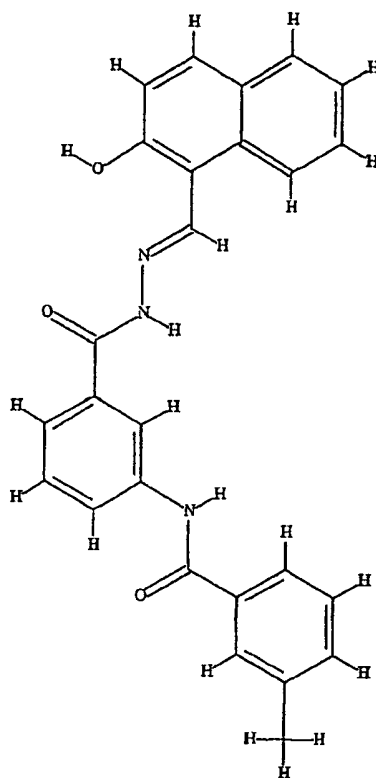
312



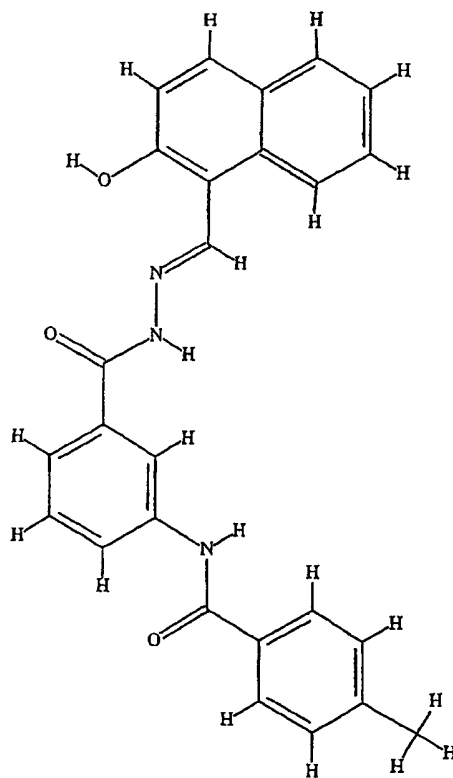
SUBSTITUTE SHEET (RULE 26)

157/248

313



314

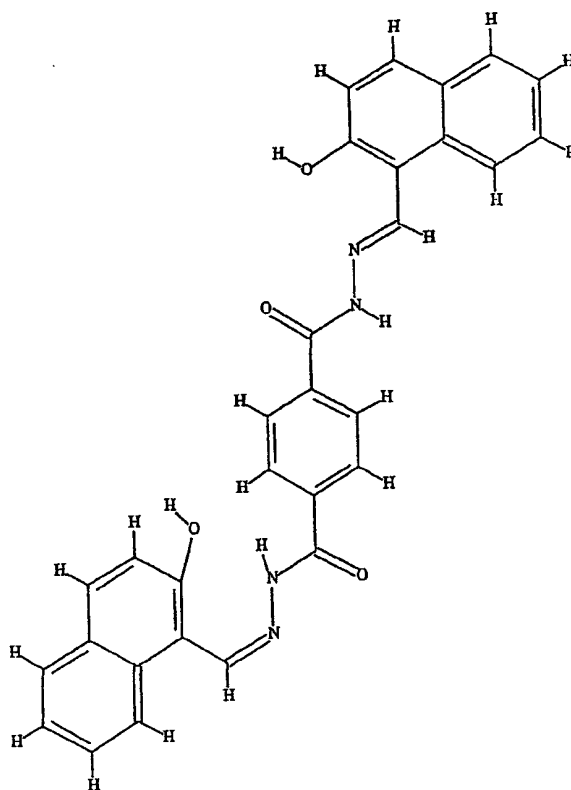


SUBSTITUTE SHEET (RULE 26)

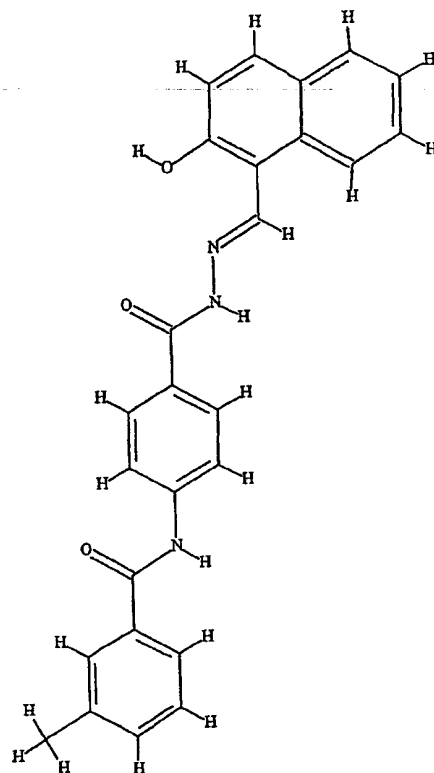


158/248

315

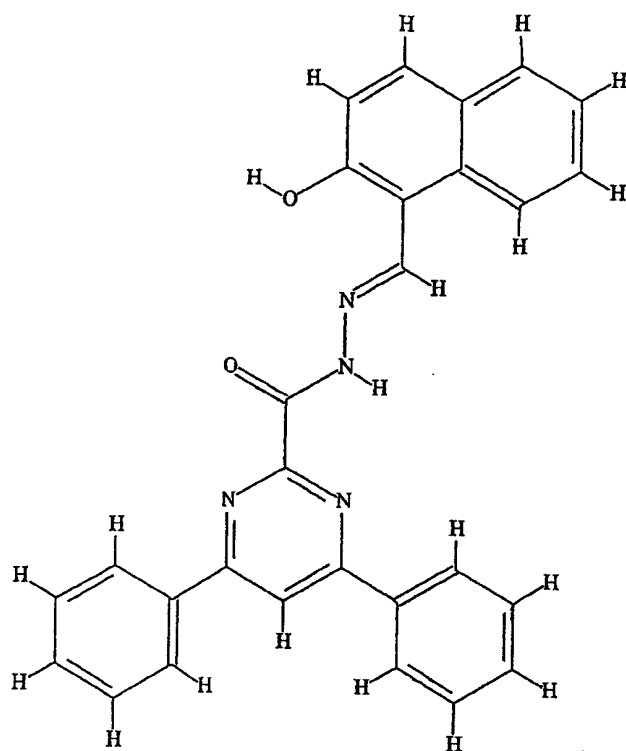


316

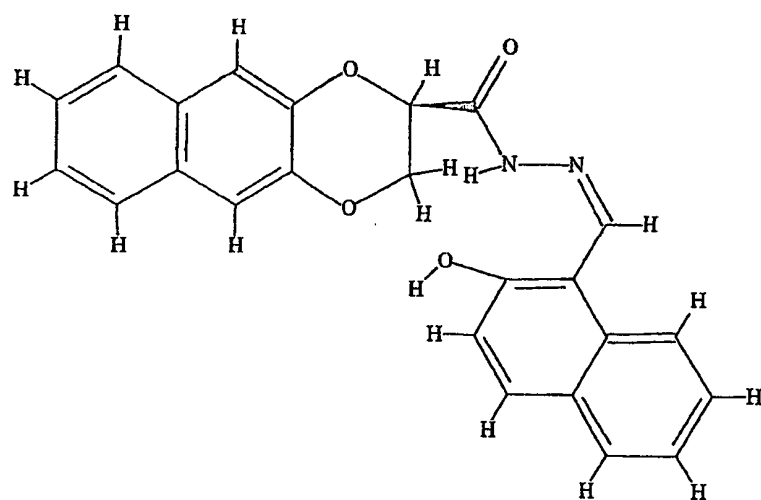


SUBSTITUTE SHEET (RULE 26)

317

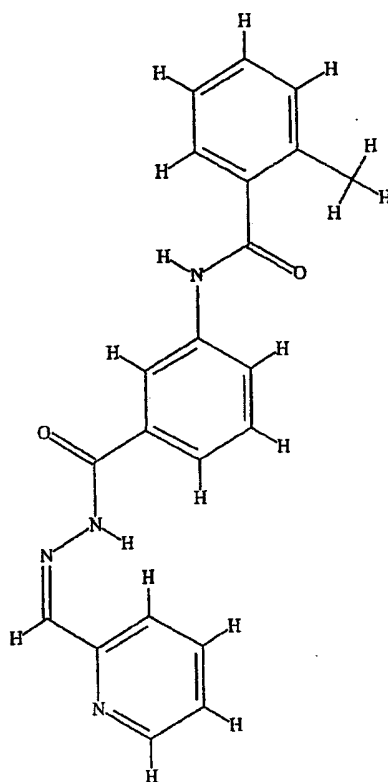


318

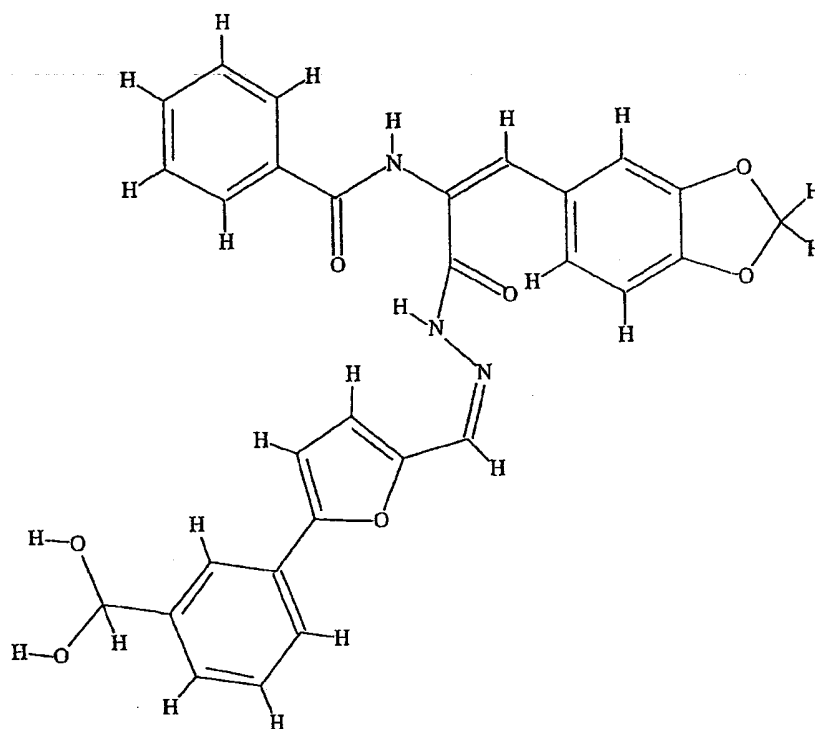


160/248

319



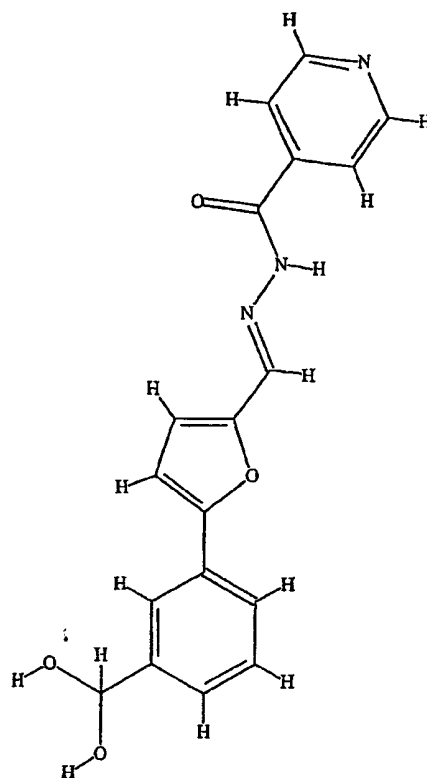
320



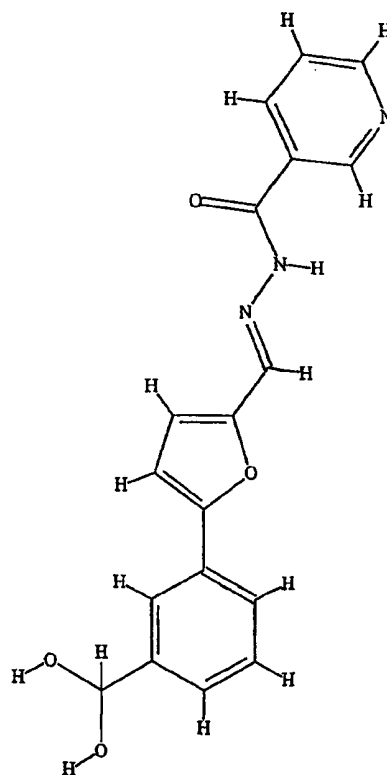
SUBSTITUTE SHEET (RULE 26)

161/248

321

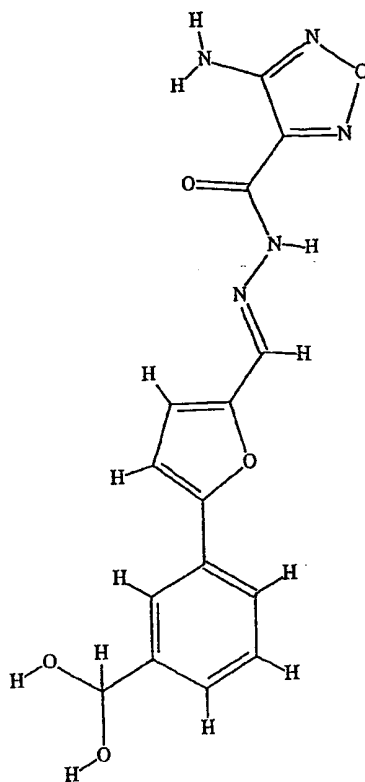


322

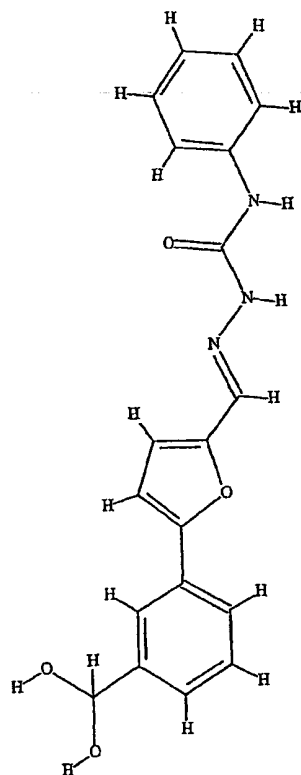


SUBSTITUTE SHEET (RULE 26)

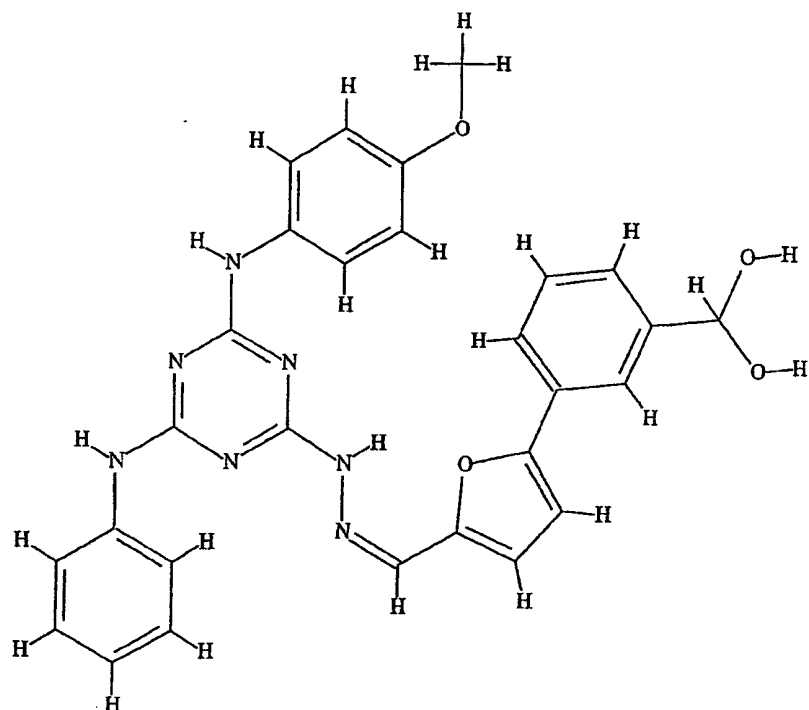
323



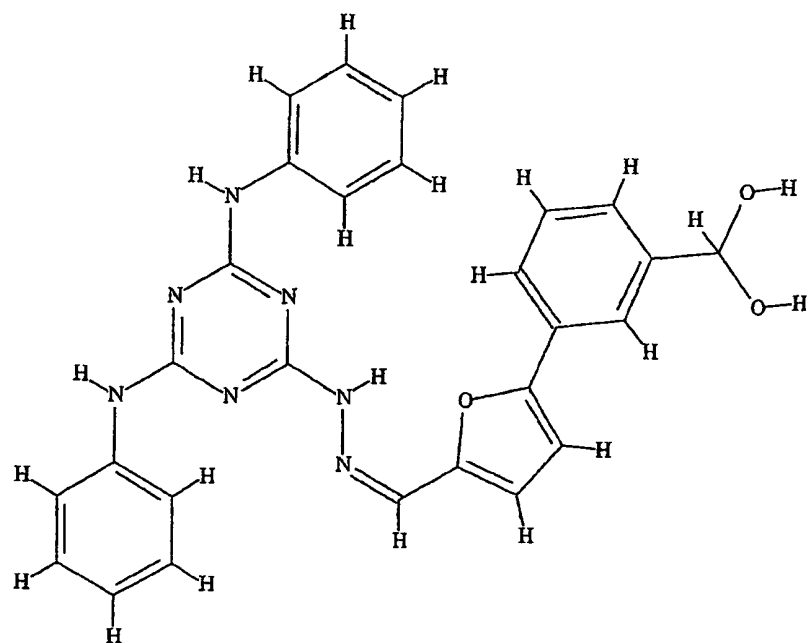
324



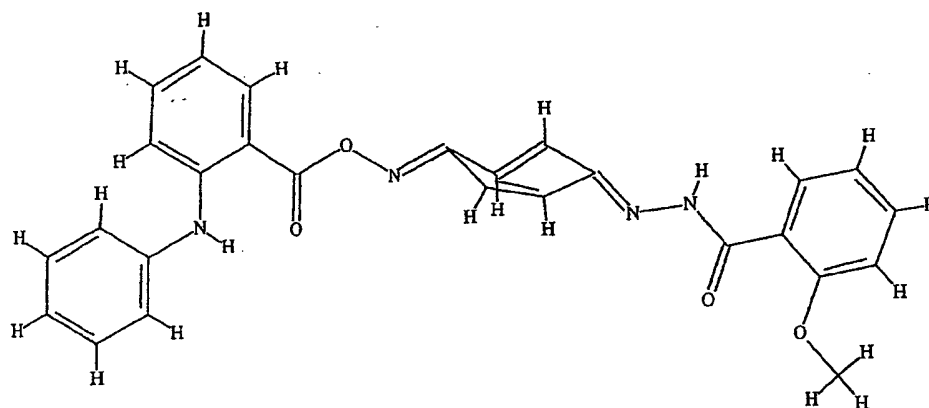
SUBSTITUTE SHEET (RULE 26)



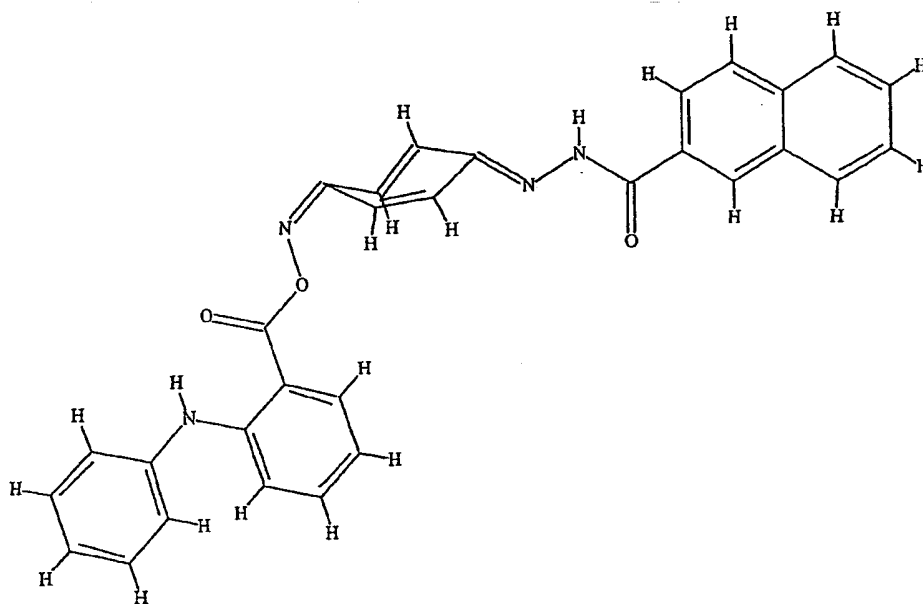
326



327

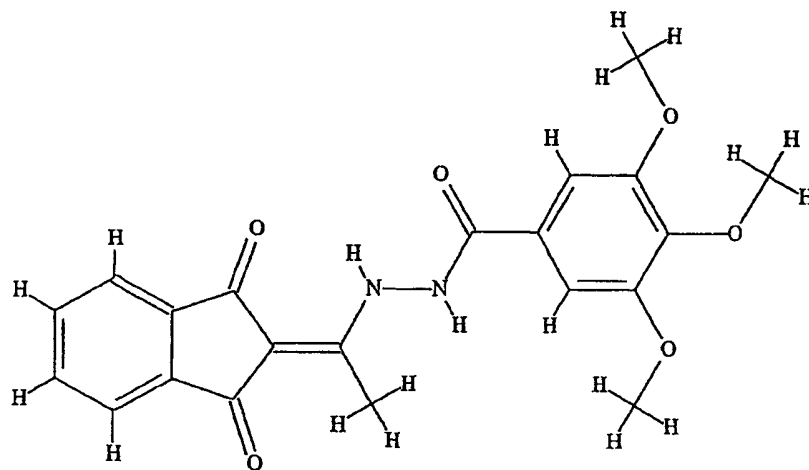


328

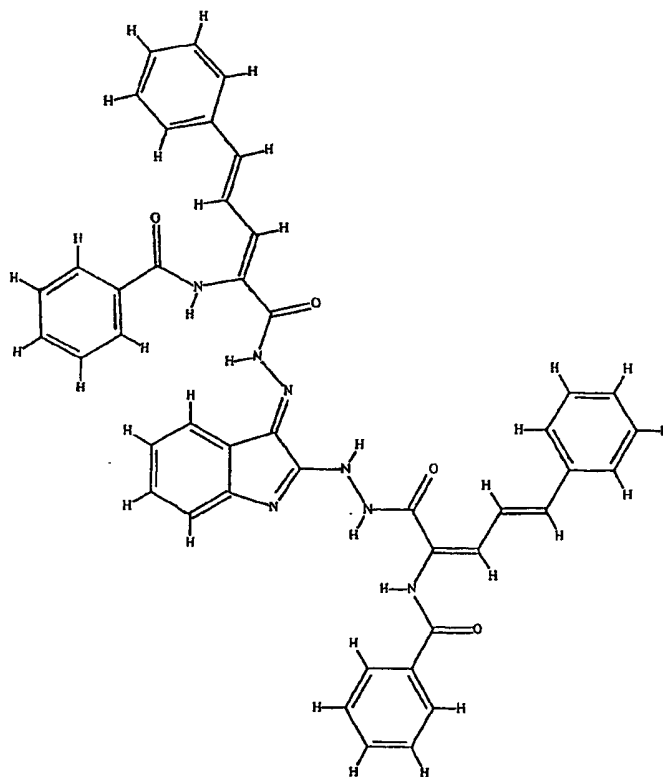


SUBSTITUTE SHEET (RULE 26)

329

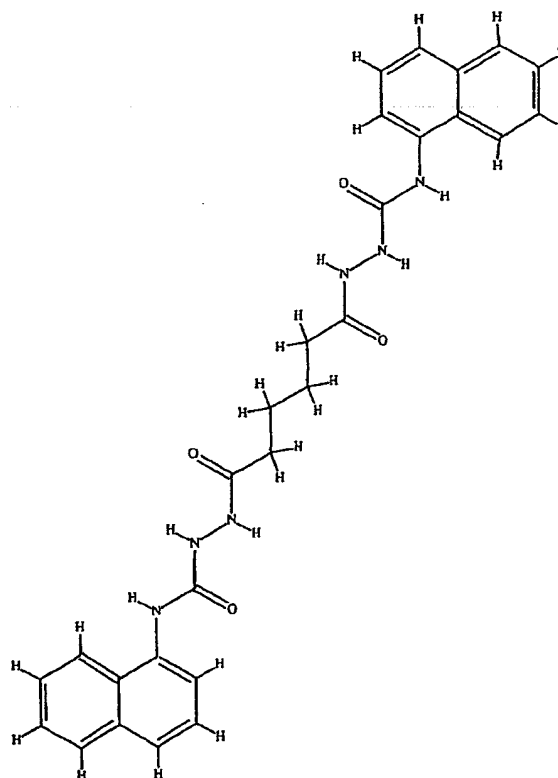
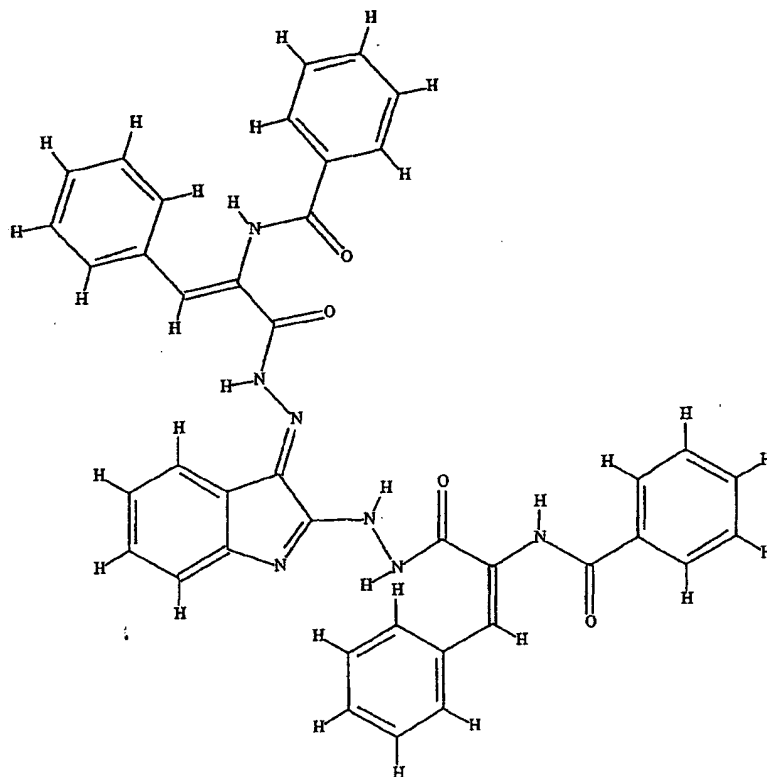


330



SUBSTITUTE SHEET (RULE 26)

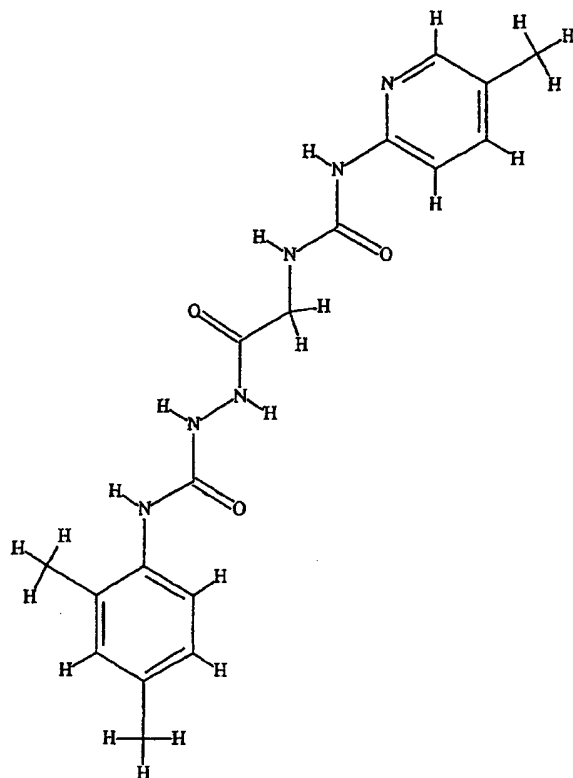




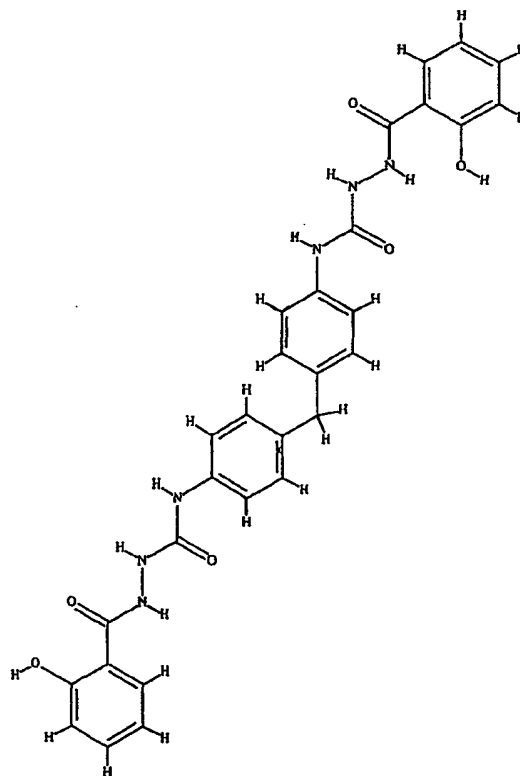
**SUBSTITUTE SHEET (RULE 26)**

167/248

333



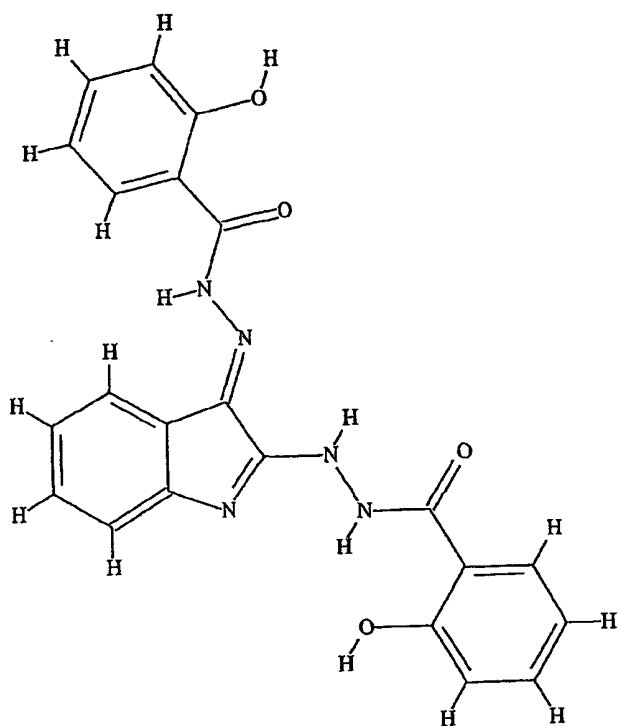
334



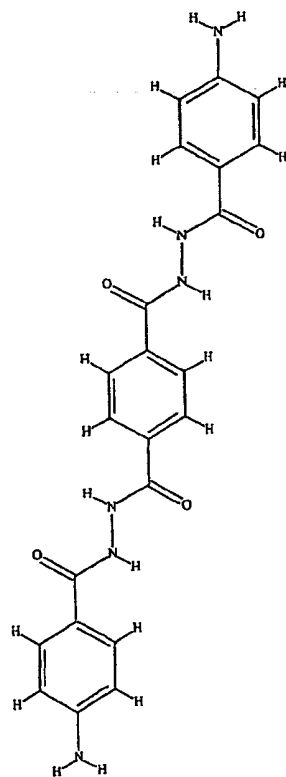
SUBSTITUTE SHEET (RULE 26)

168/248

335



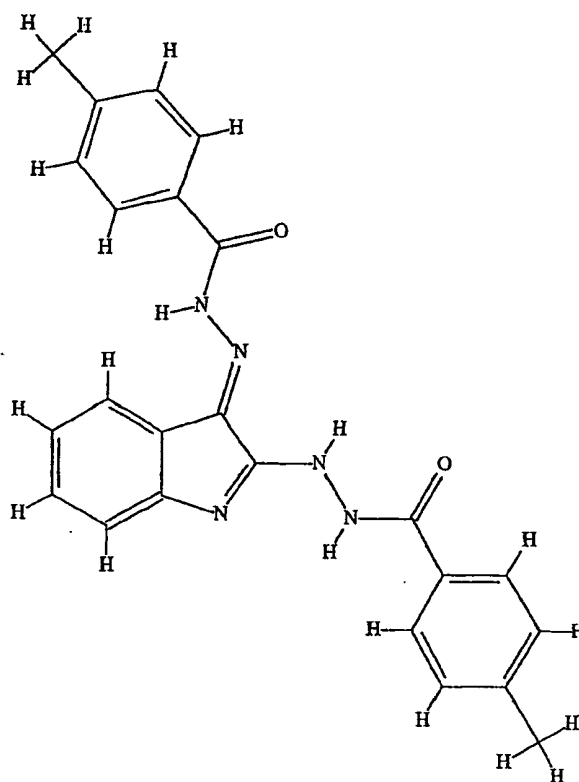
336



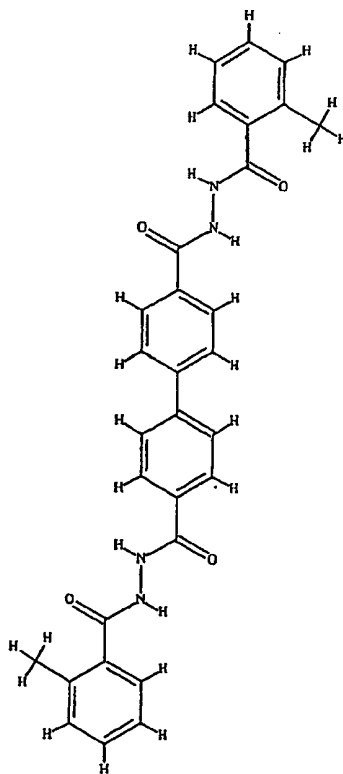
SUBSTITUTE SHEET (RULE 26)

169/248

337



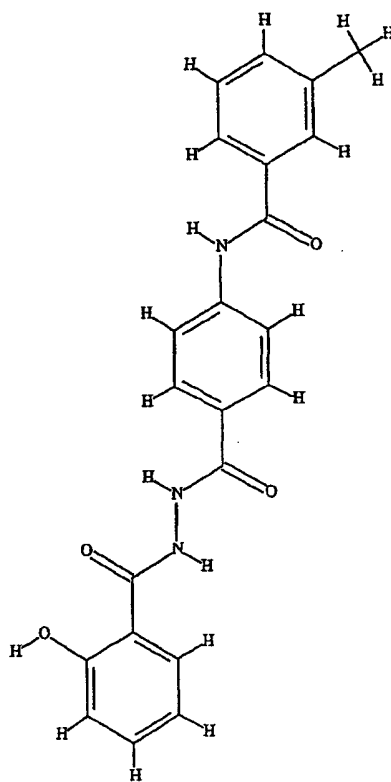
338



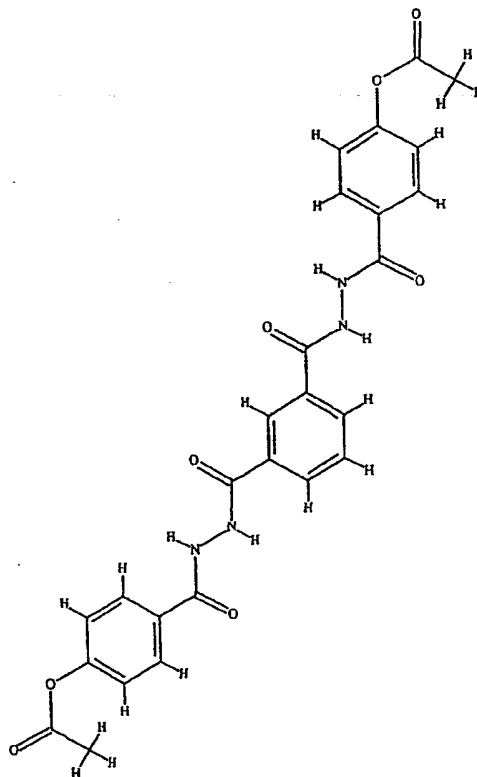
SUBSTITUTE SHEET (RULE 26)

170/248

339

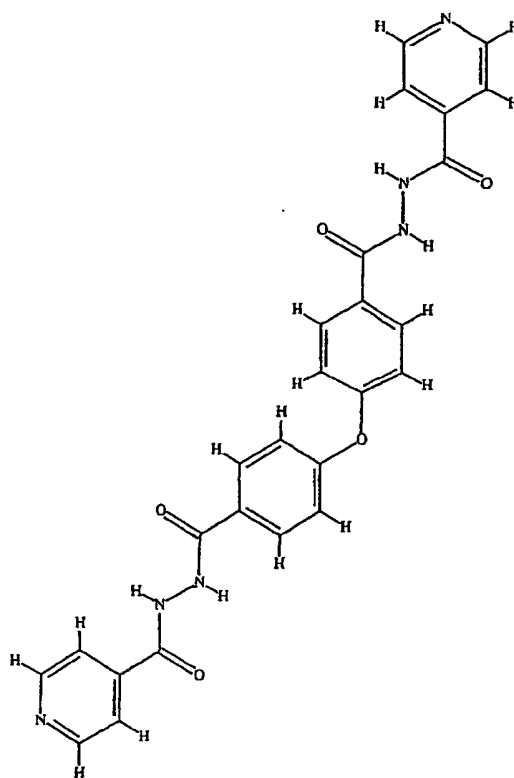


340

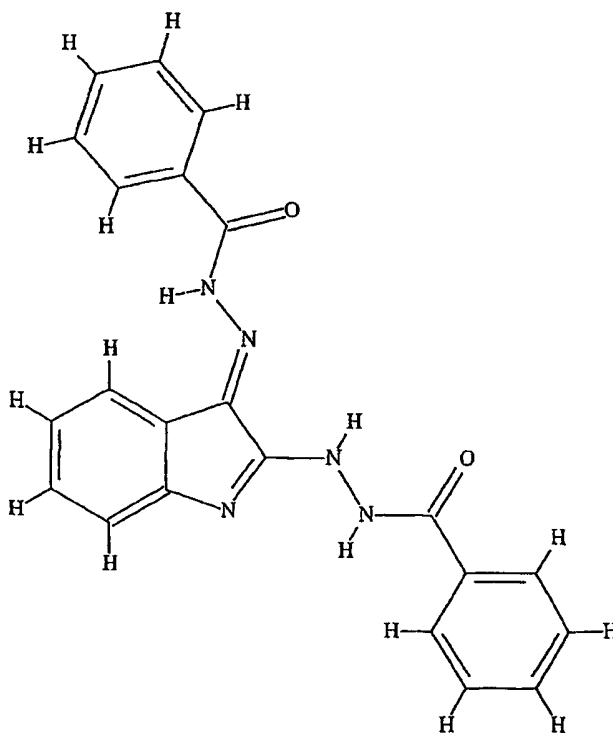


SUBSTITUTE SHEET (RULE 26)

341

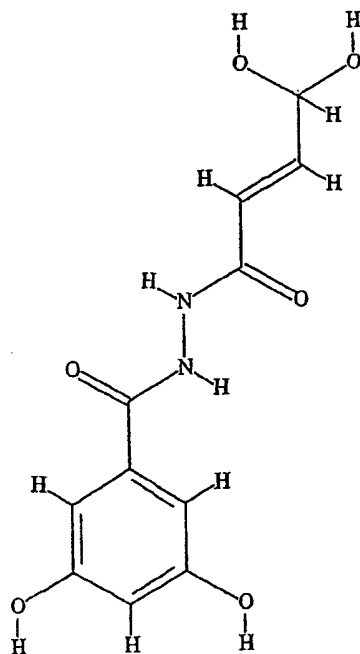


342

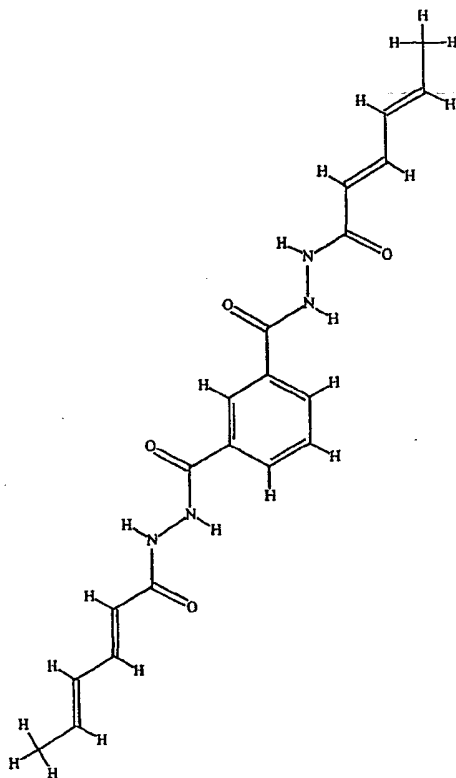


172/248

343

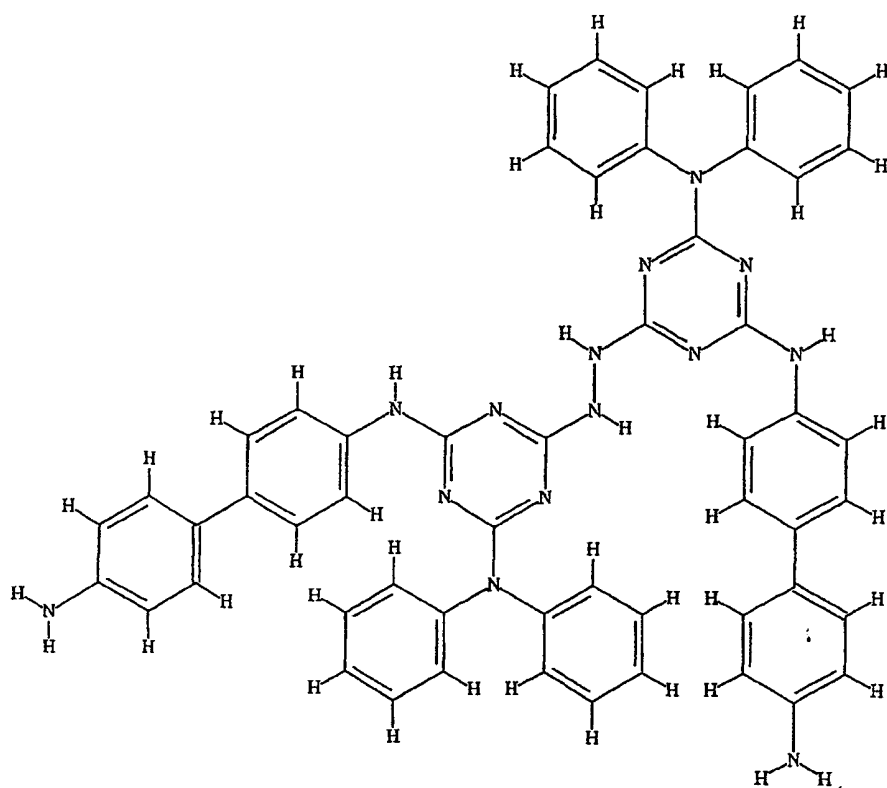


344

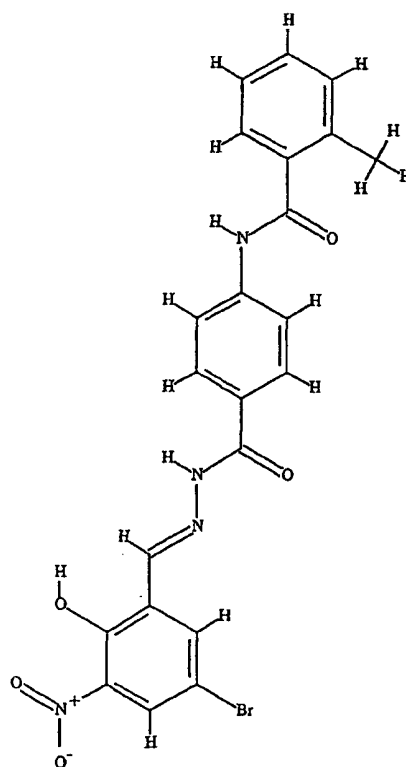


SUBSTITUTE SHEET (RULE 26)

345



346

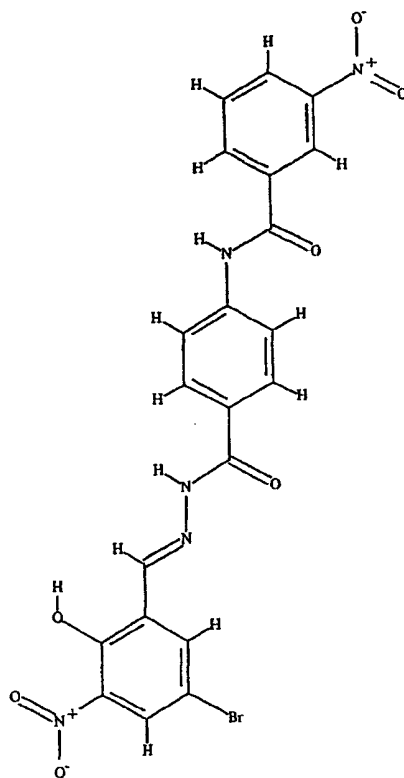


SUBSTITUTE SHEET (RULE 26)

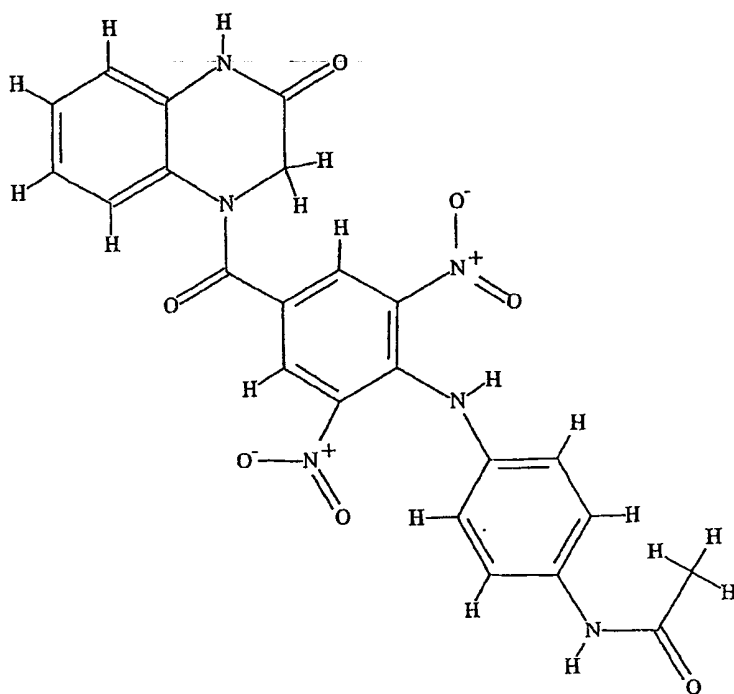


174/248

347



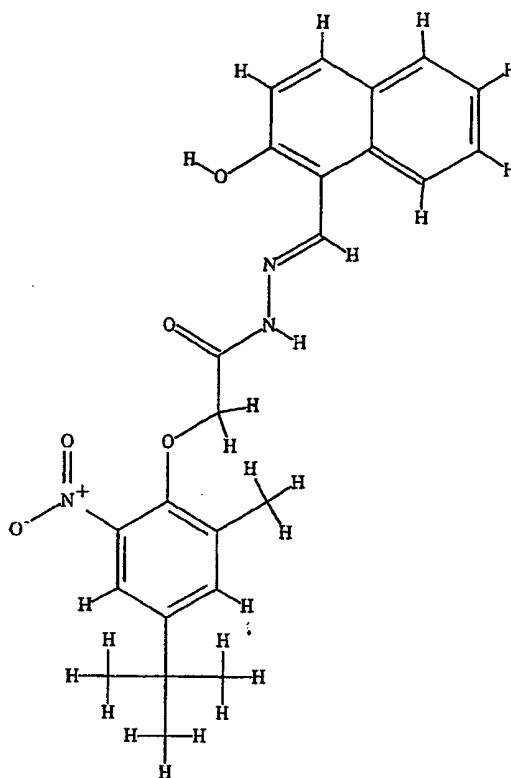
348



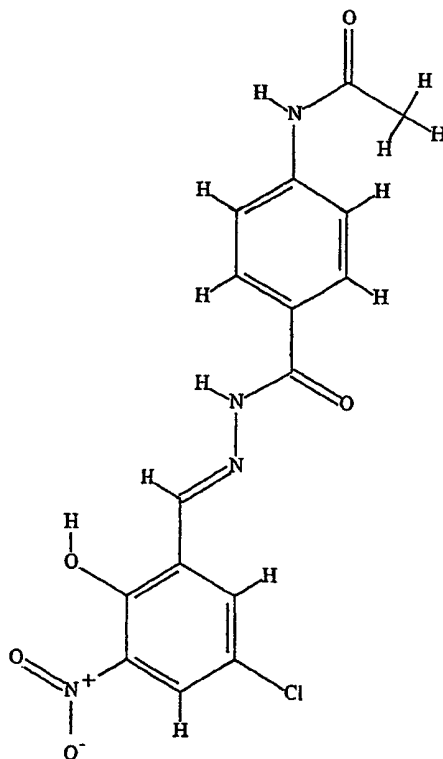
SUBSTITUTE SHEET (RULE 26)

175/248

349



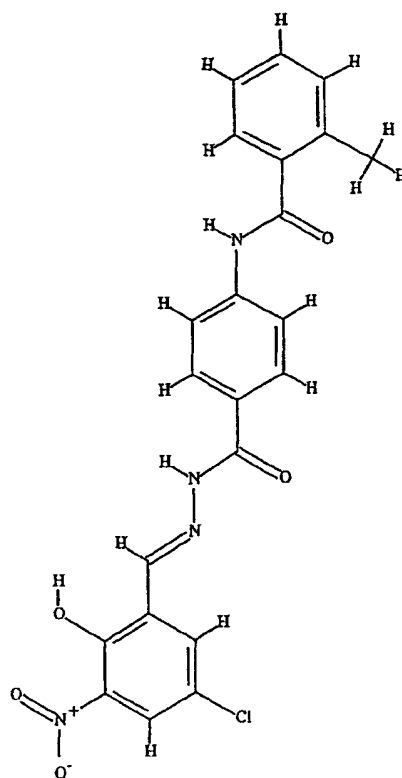
350



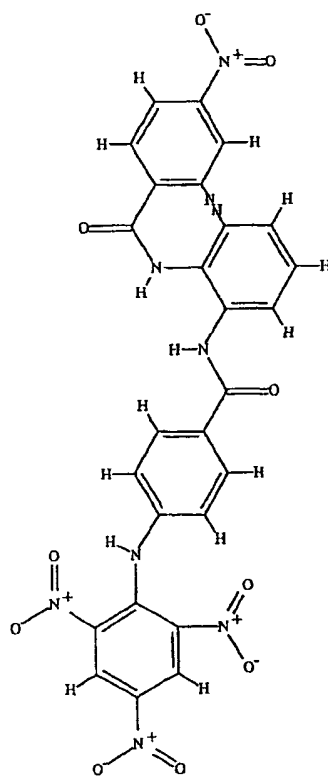
SUBSTITUTE SHEET (RULE 26)

176/248

351

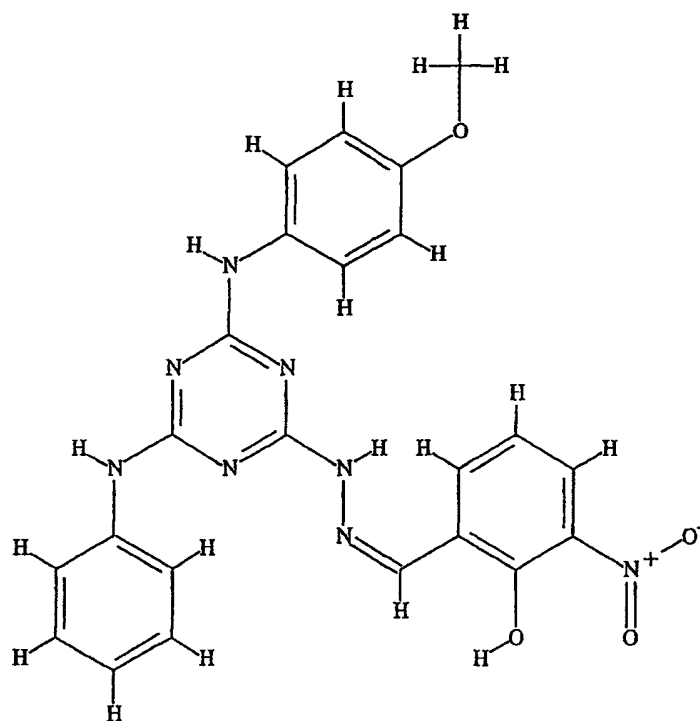


352

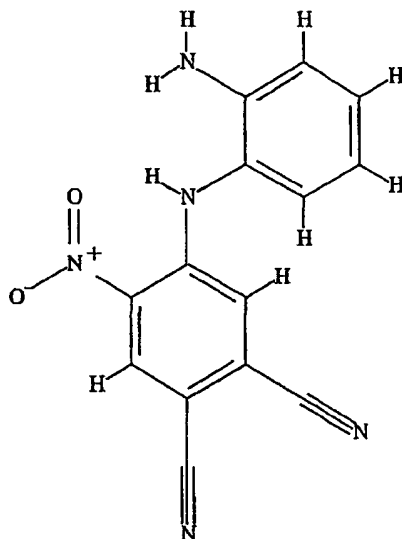


SUBSTITUTE SHEET (RULE 26)

353



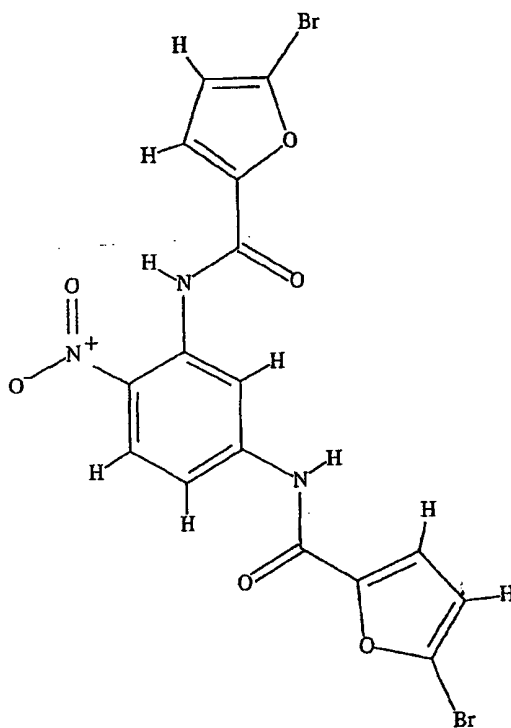
354



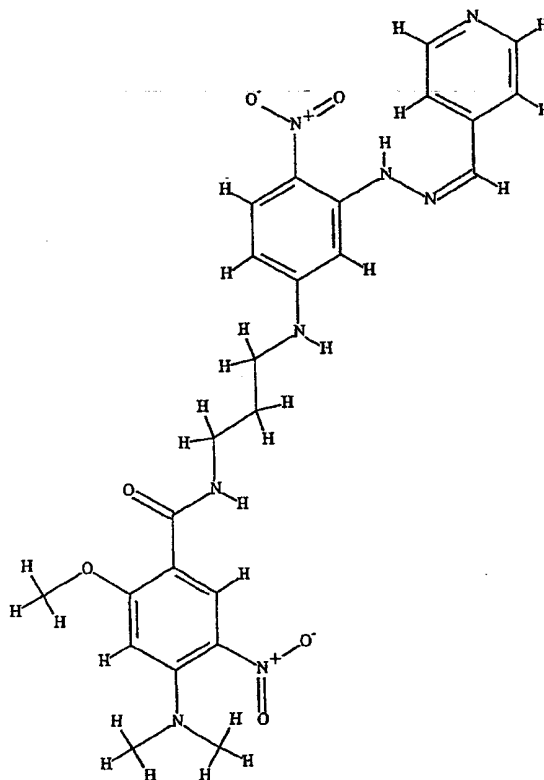
SUBSTITUTE SHEET (RULE 26)

178/248

355

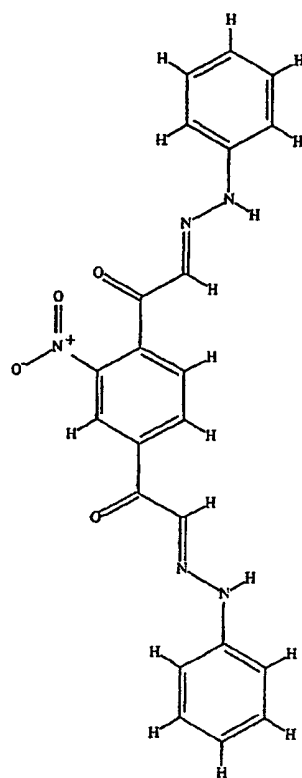


356

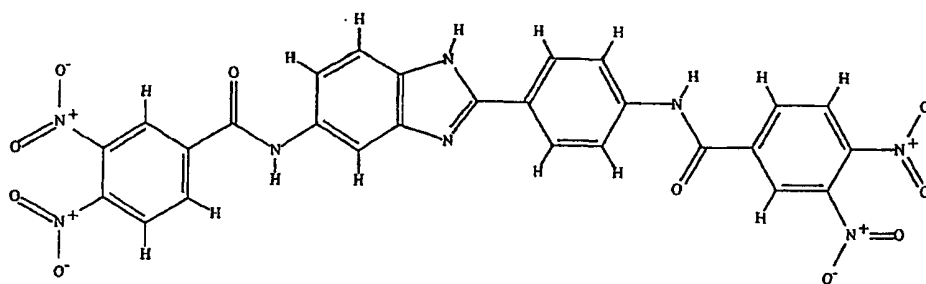


SUBSTITUTE SHEET (RULE 26)

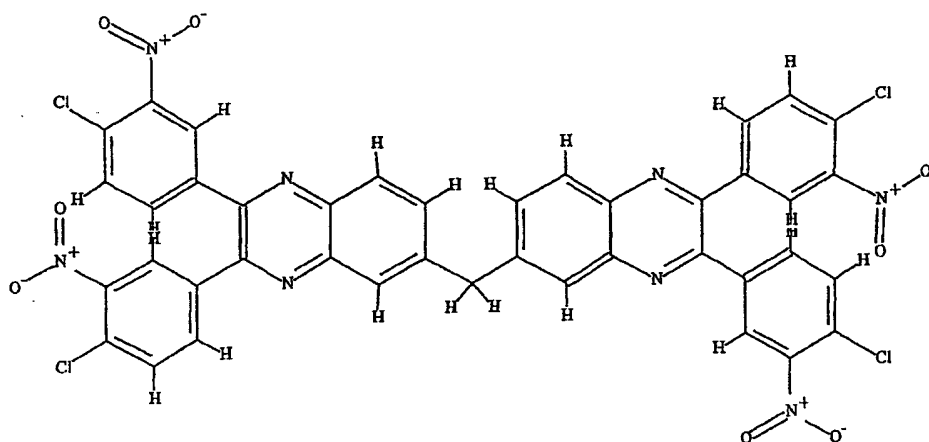
357



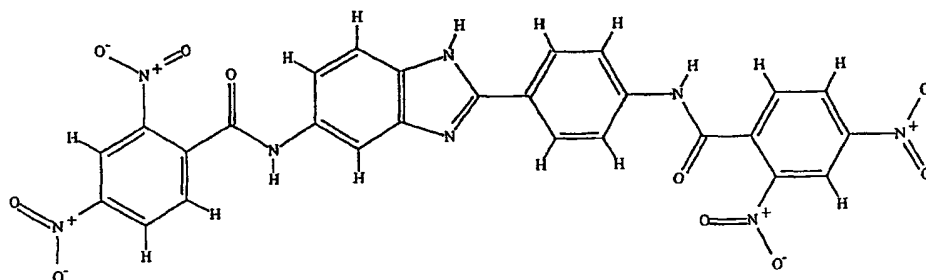
358



359



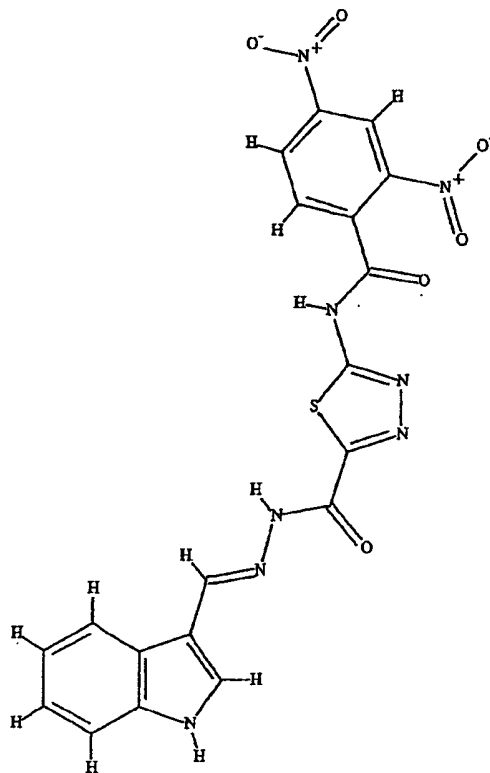
360



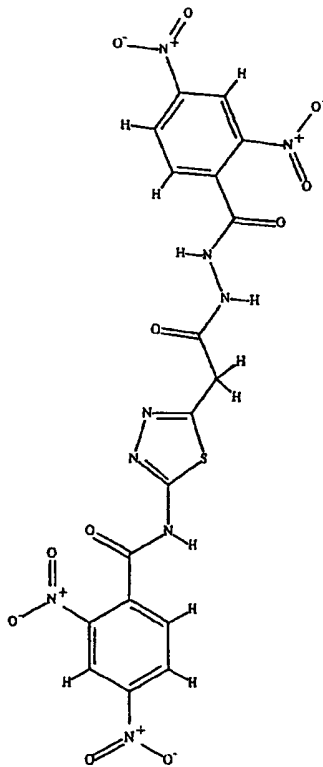
SUBSTITUTE SHEET (RULE 26)

181/248

361



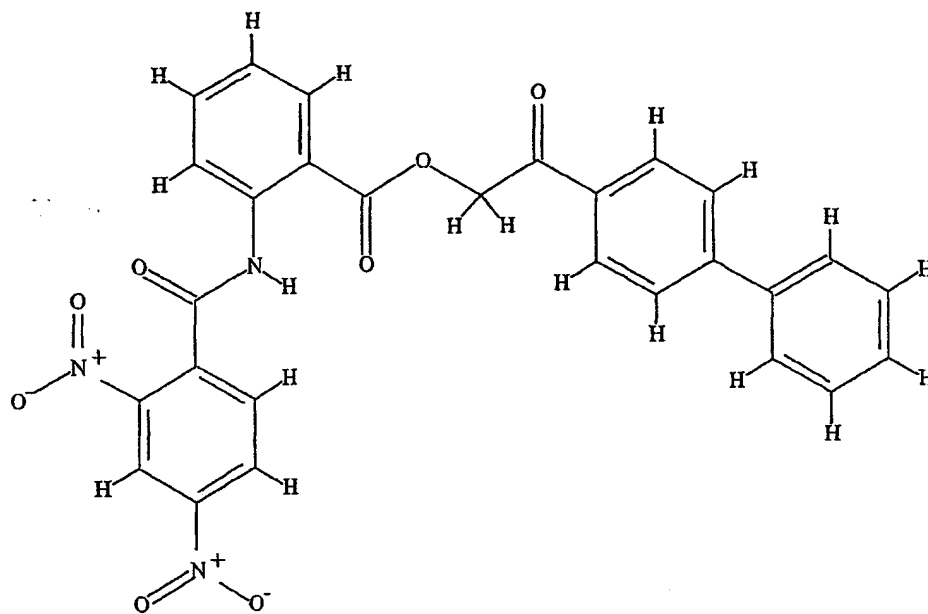
362



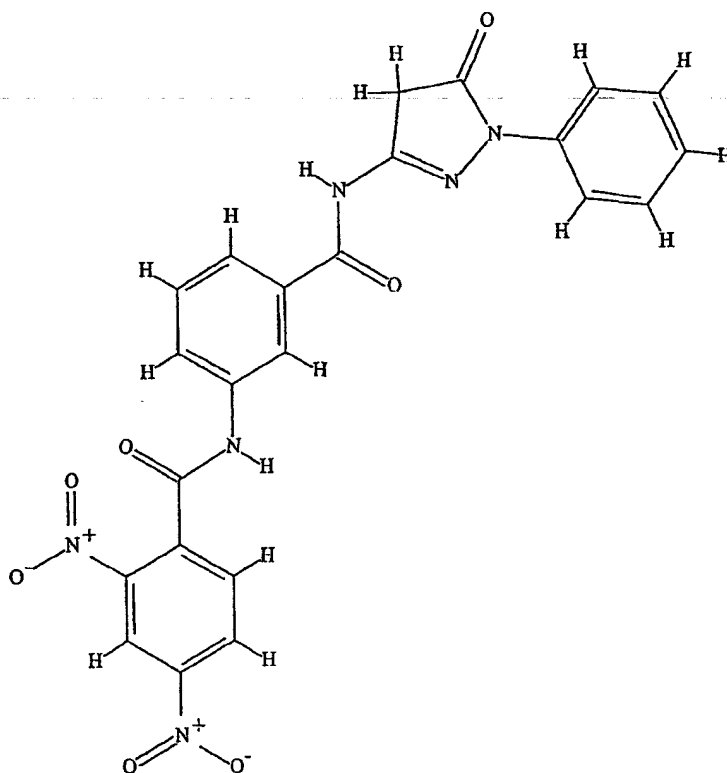
SUBSTITUTE SHEET (RULE 26)



363



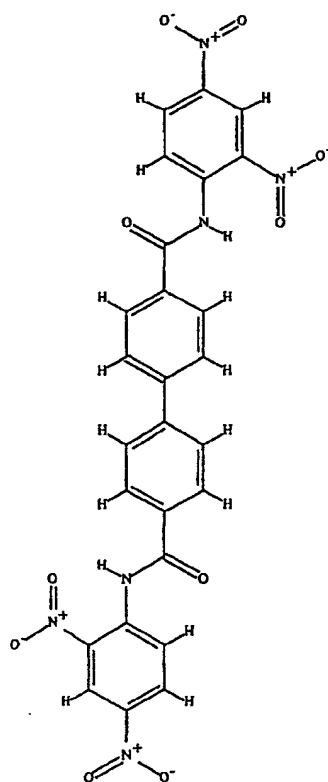
364



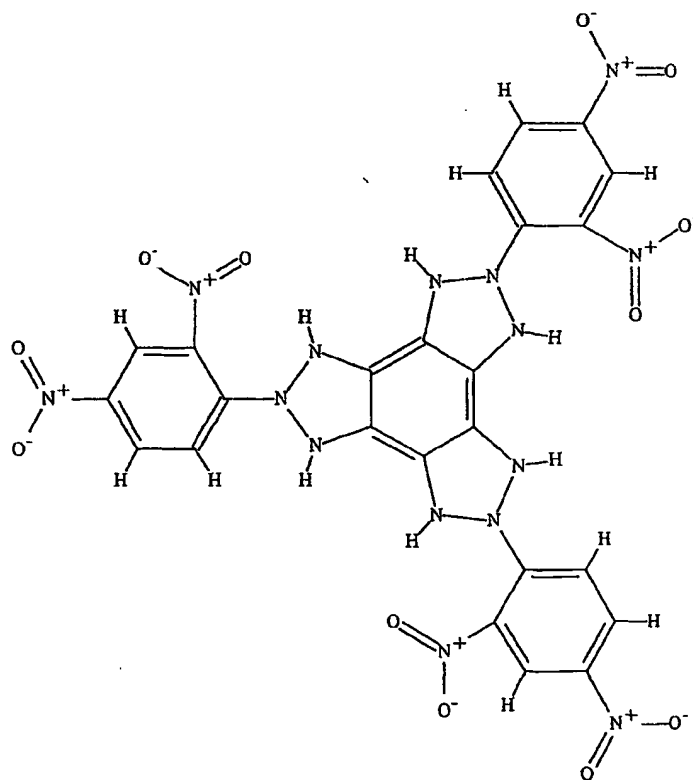
SUBSTITUTE SHEET (RULE 26)

183/248

365

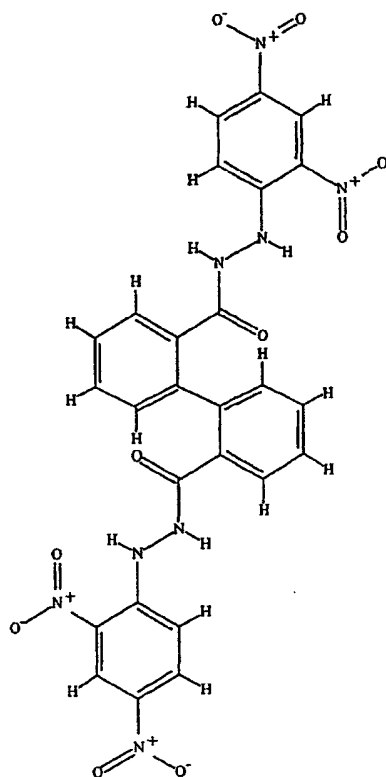


366

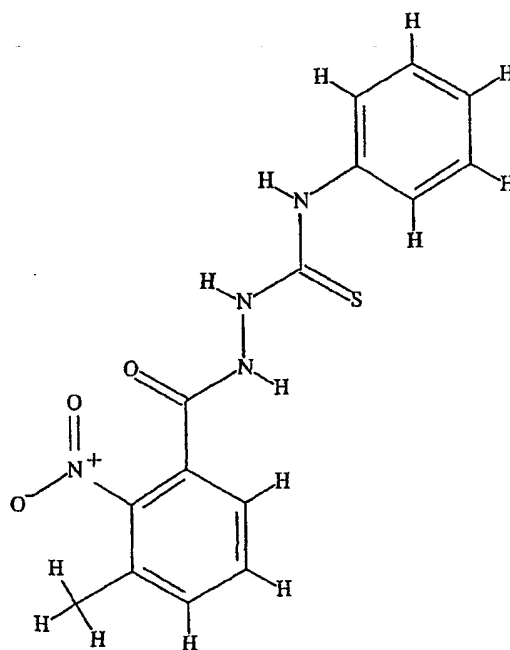


SUBSTITUTE SHEET (RULE 26)

367



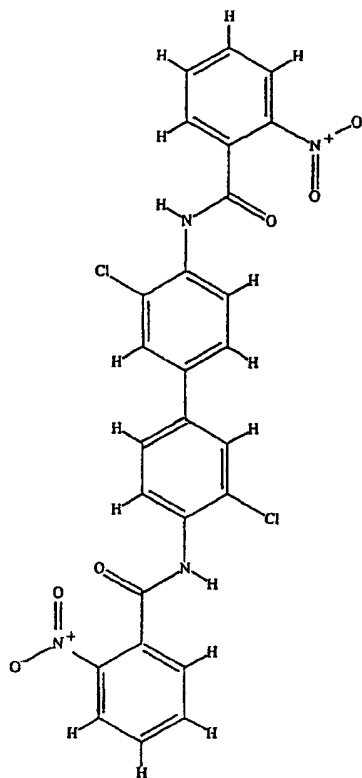
368



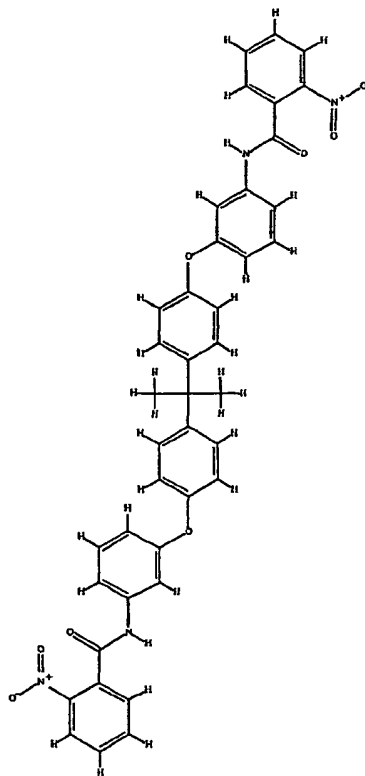
SUBSTITUTE SHEET (RULE 26)

185/248

369

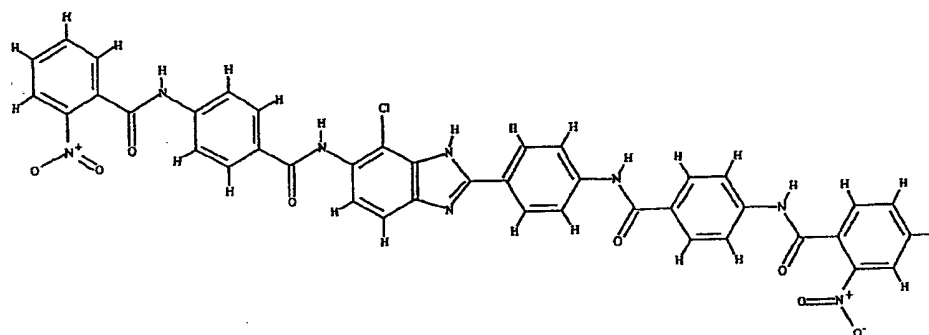


370

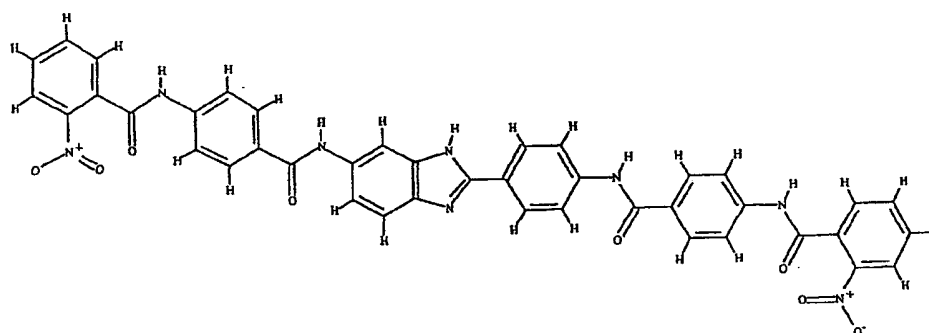


SUBSTITUTE SHEET (RULE 26)

371

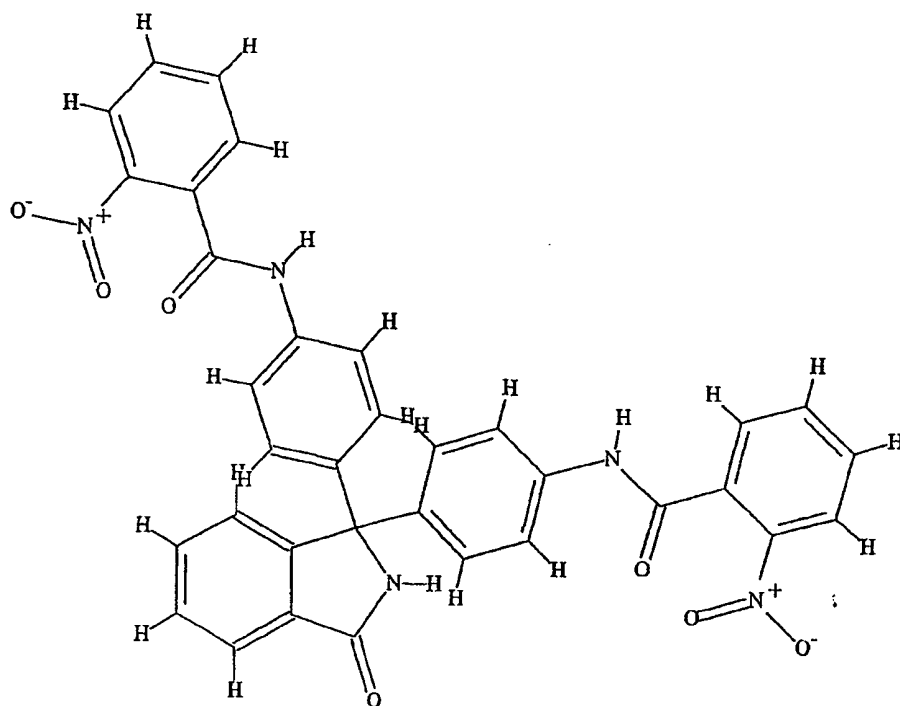


372

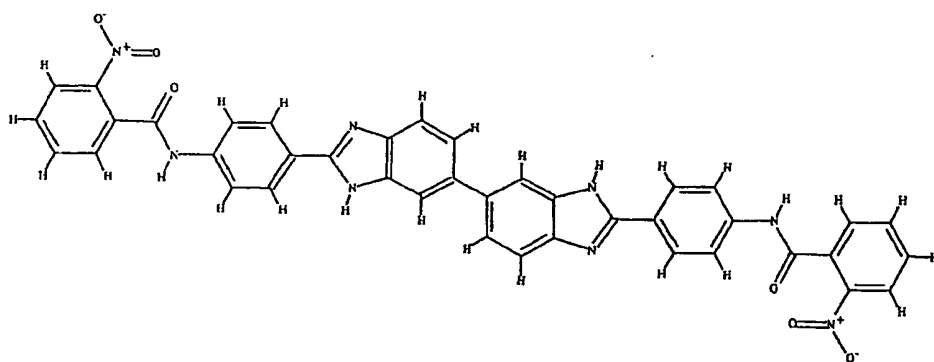


SUBSTITUTE SHEET (RULE 26)

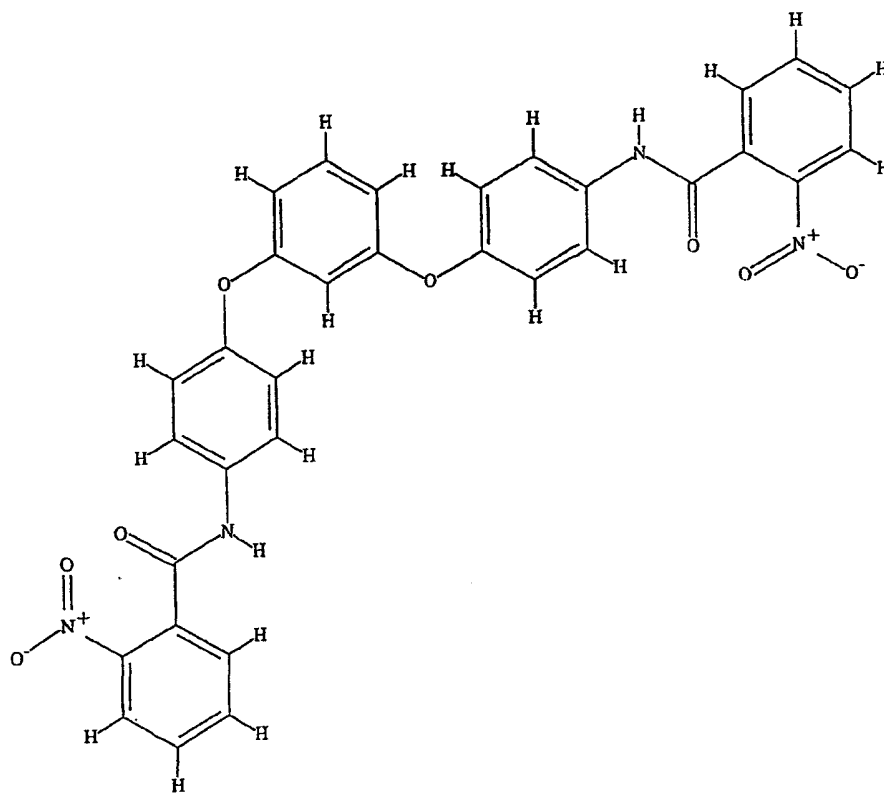
373



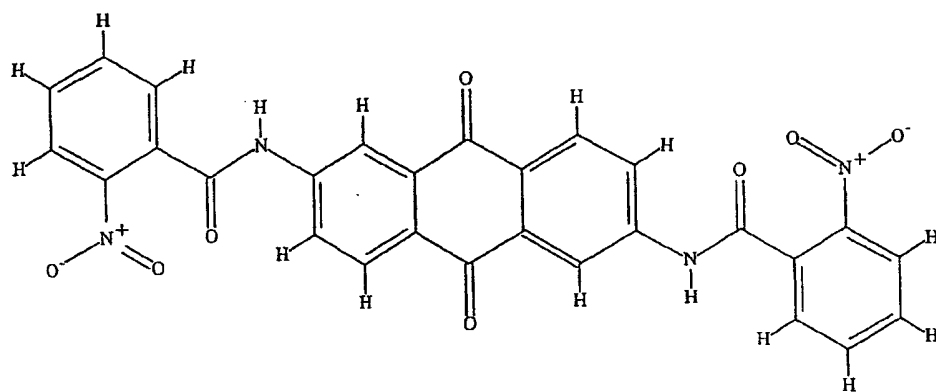
374



375

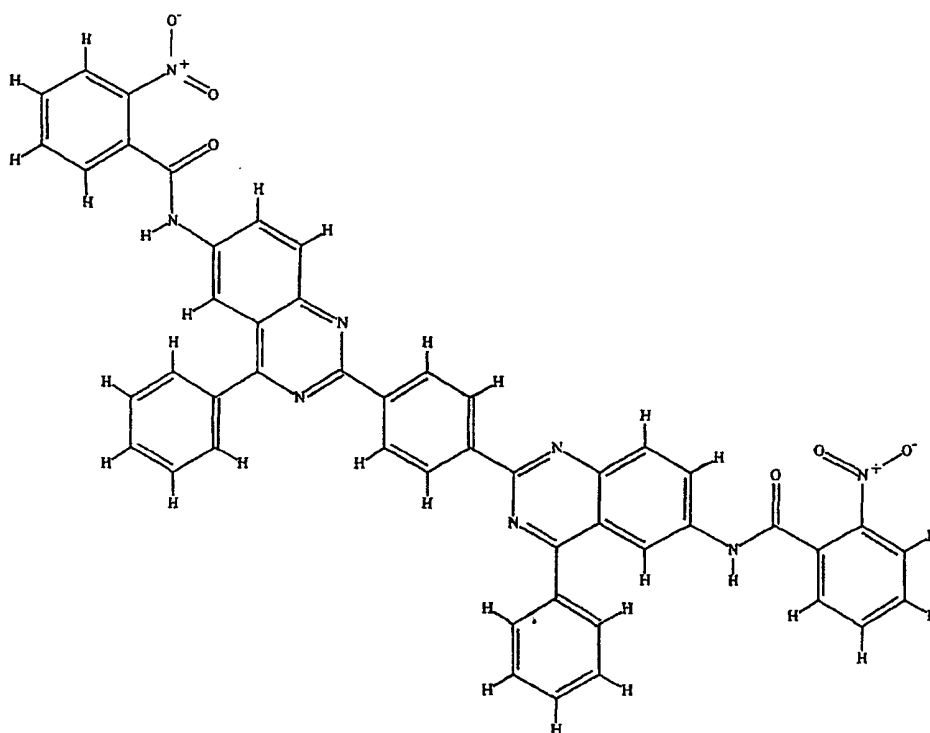


376

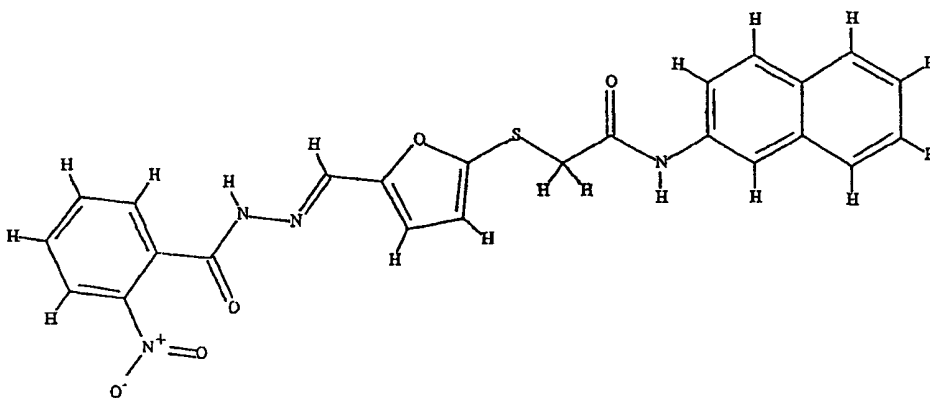


SUBSTITUTE SHEET (RULE 26)

377



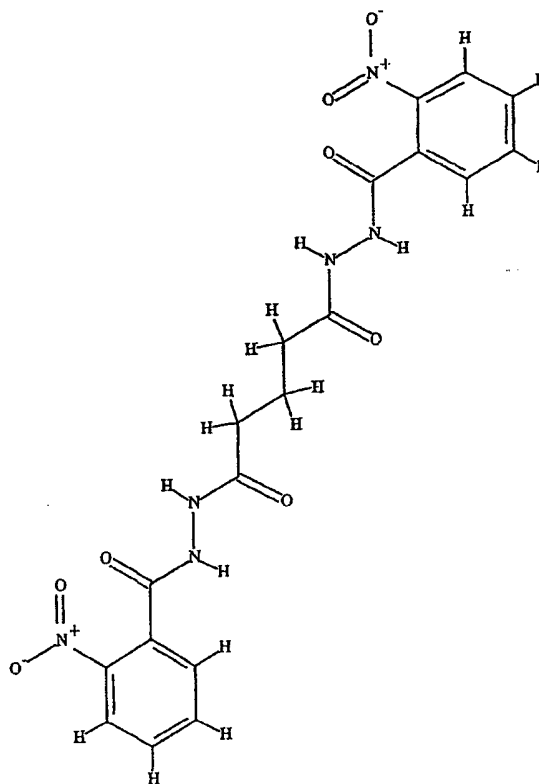
378



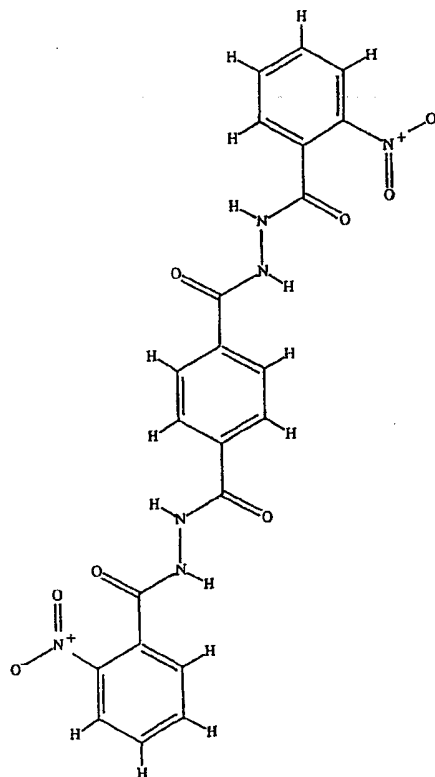
SUBSTITUTE SHEET (RULE 26)



379

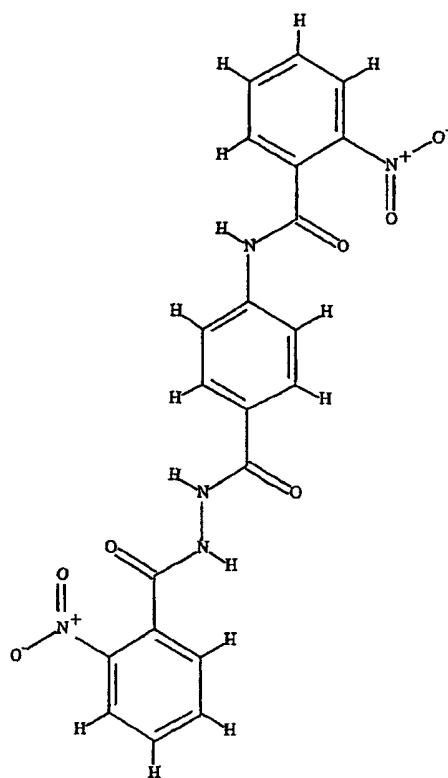


380

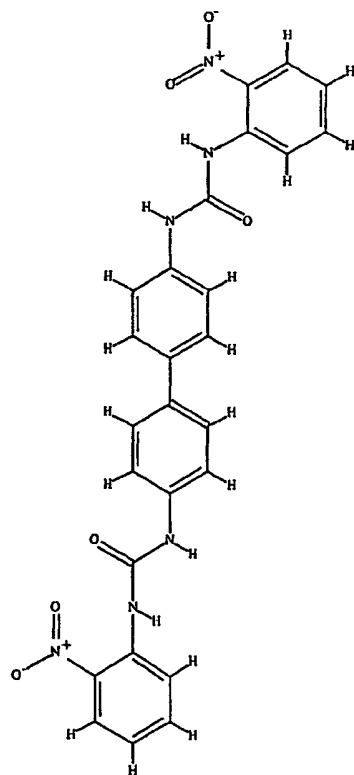


SUBSTITUTE SHEET (RULE 26)

381

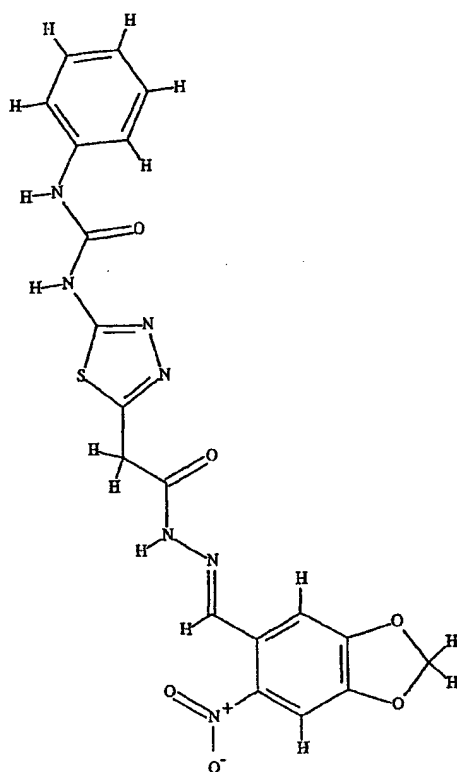


382

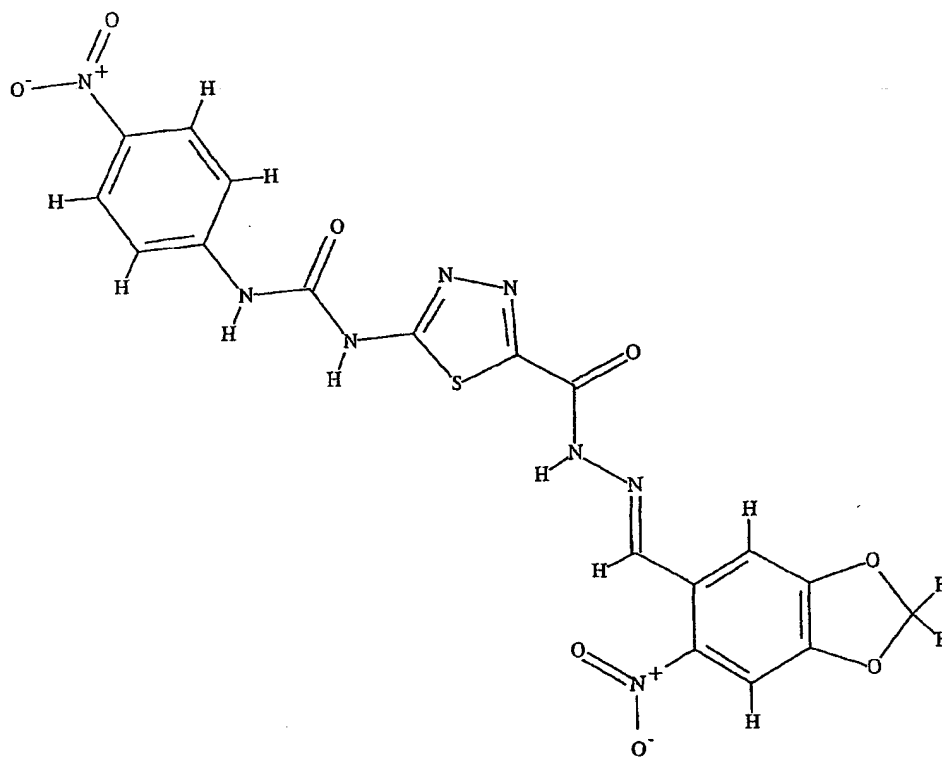


SUBSTITUTE SHEET (RULE 26)

383

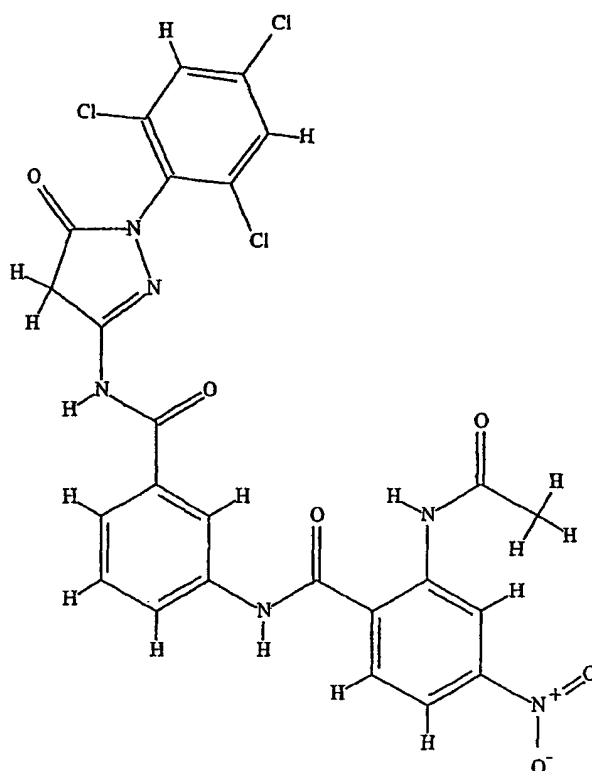


384

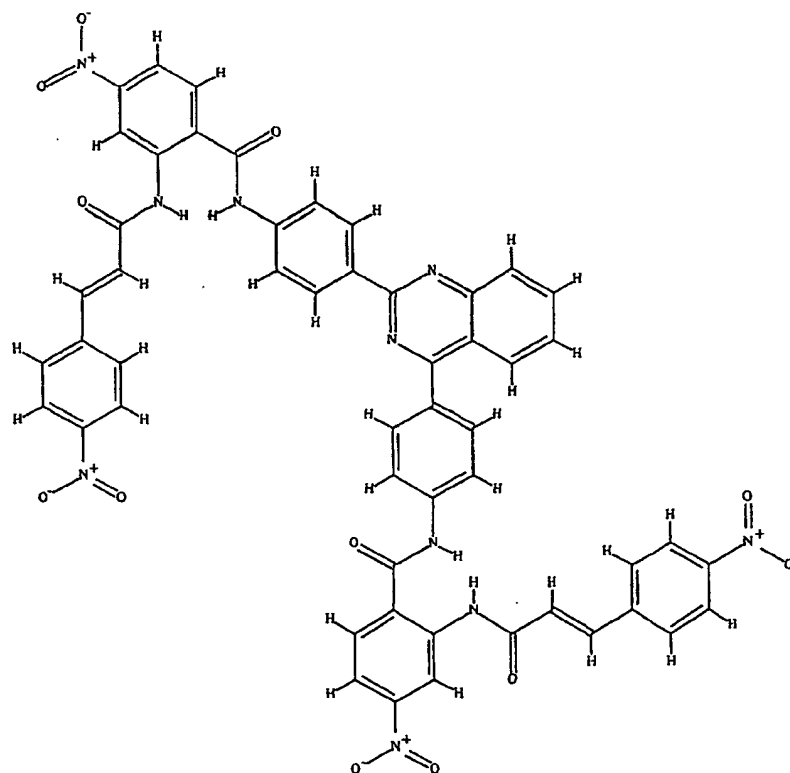


SUBSTITUTE SHEET (RULE 26)

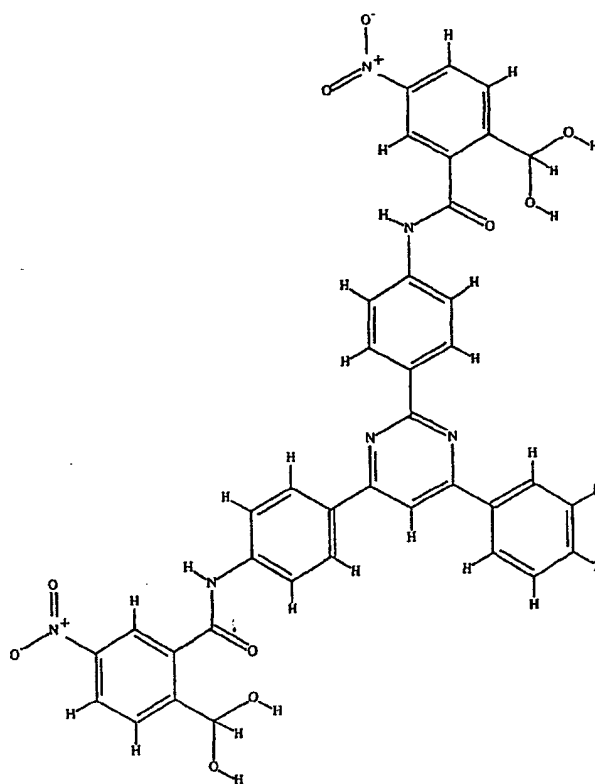
385



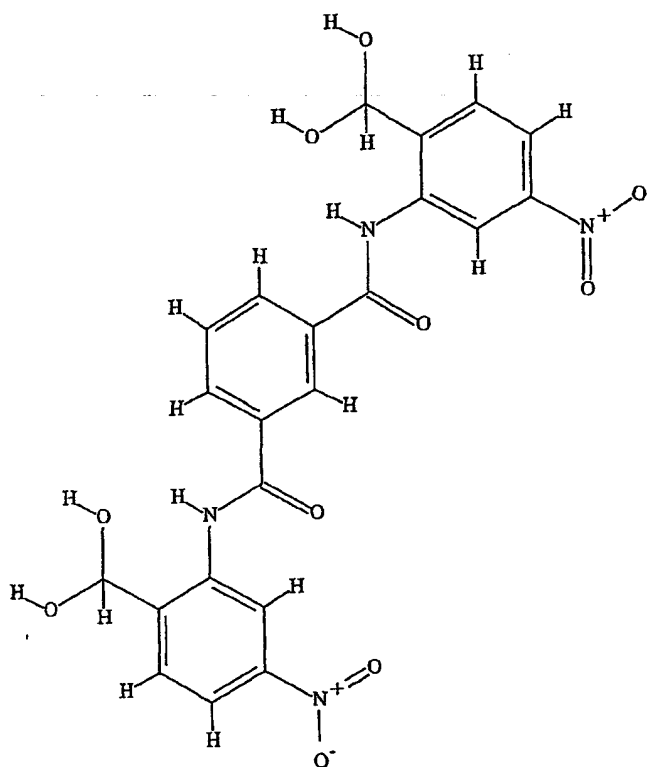
386



387

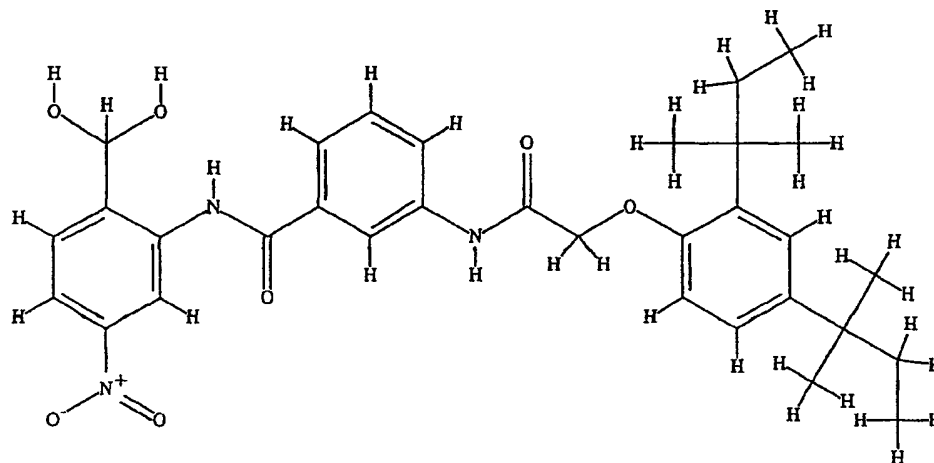


388

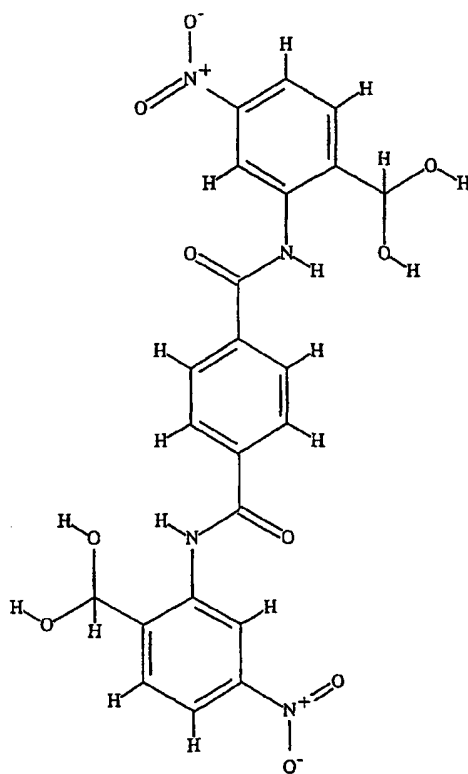


SUBSTITUTE SHEET (RULE 26)

389

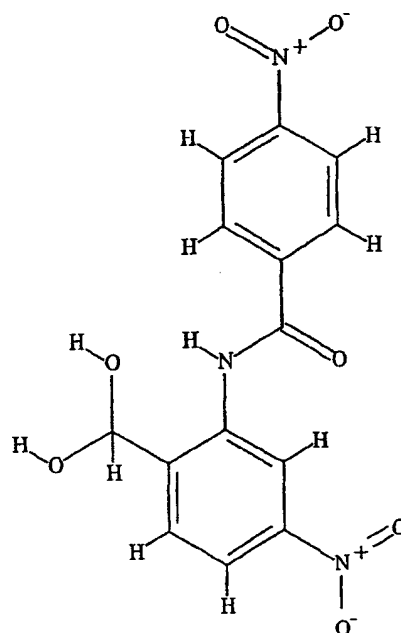


390

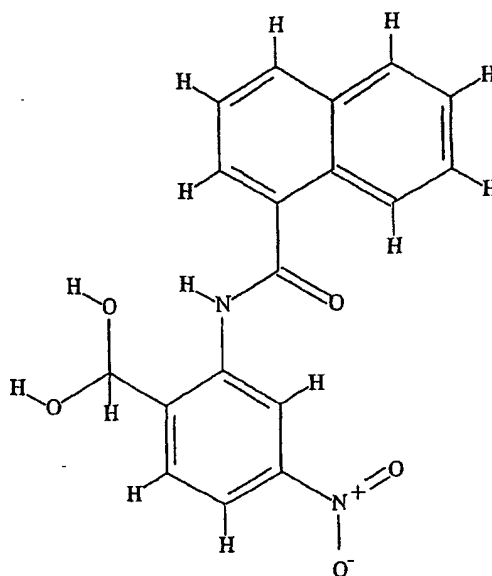


SUBSTITUTE SHEET (RULE 26)

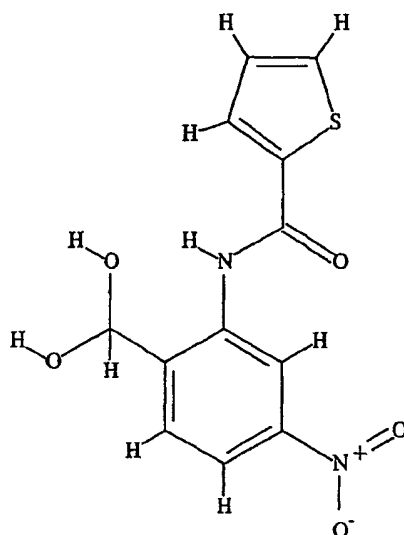
391



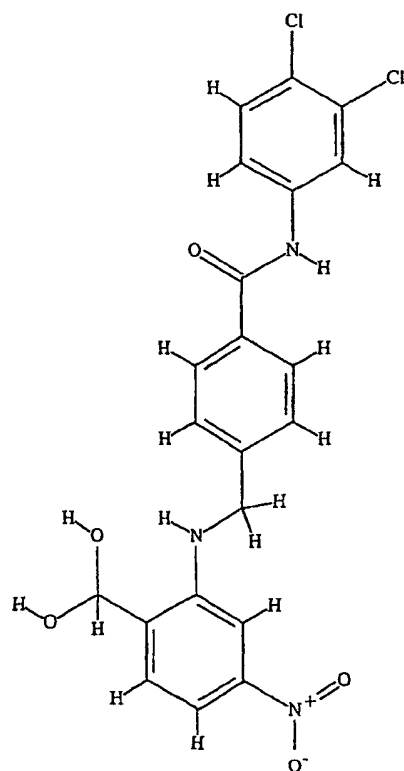
392



393



394

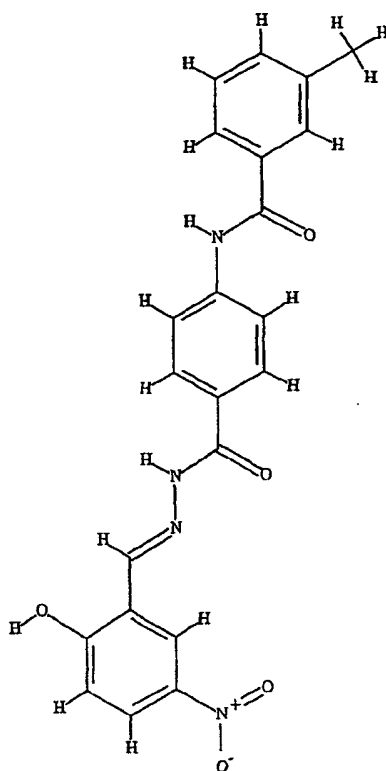


SUBSTITUTE SHEET (RULE 26)

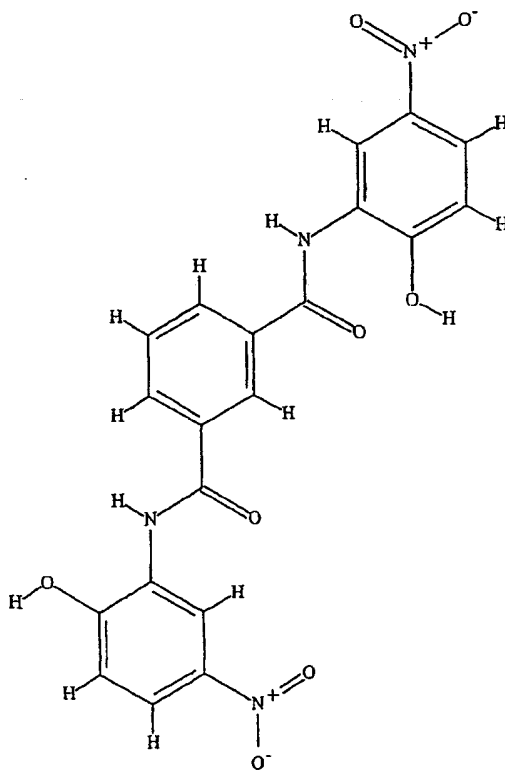


198/248

395

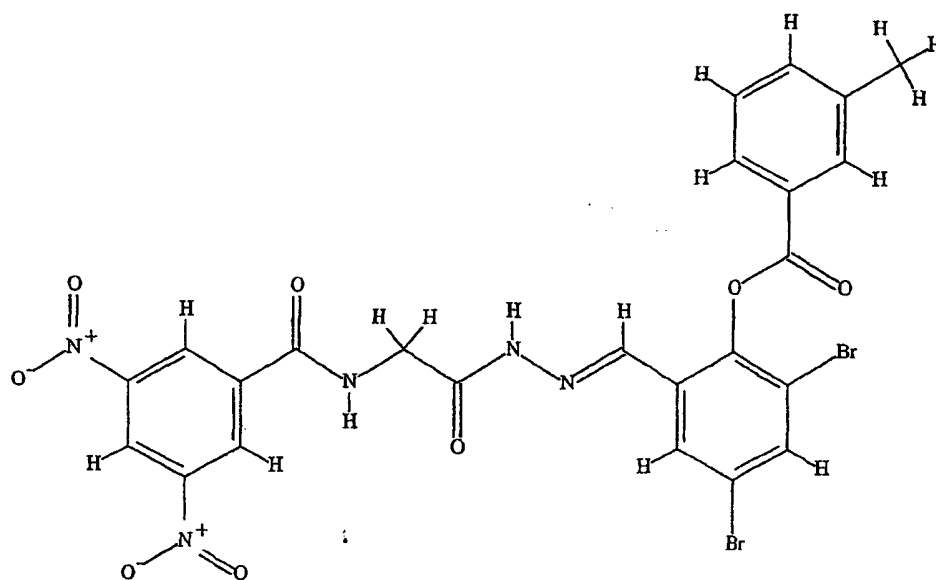


396

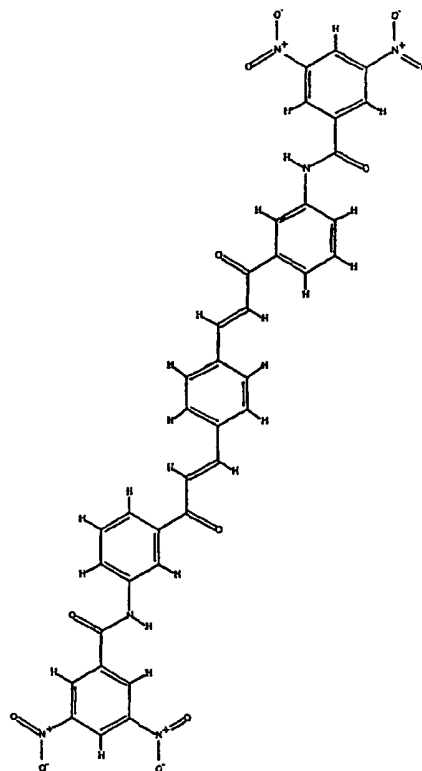


SUBSTITUTE SHEET (RULE 26)

397

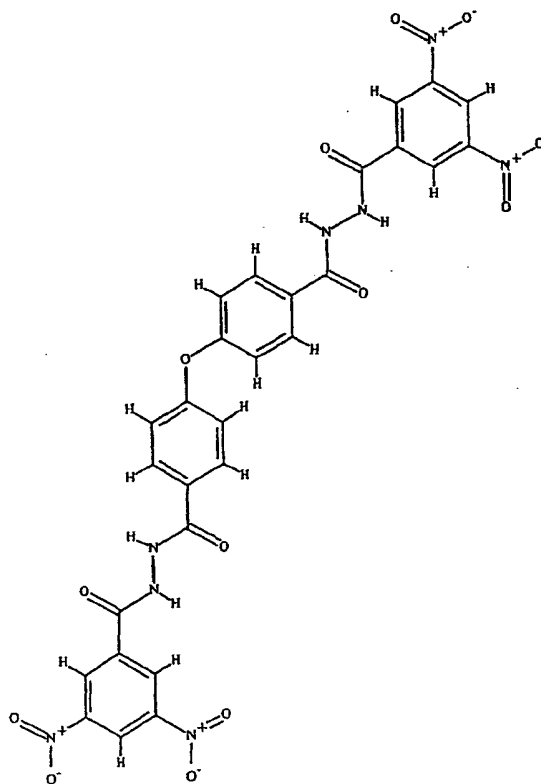


398

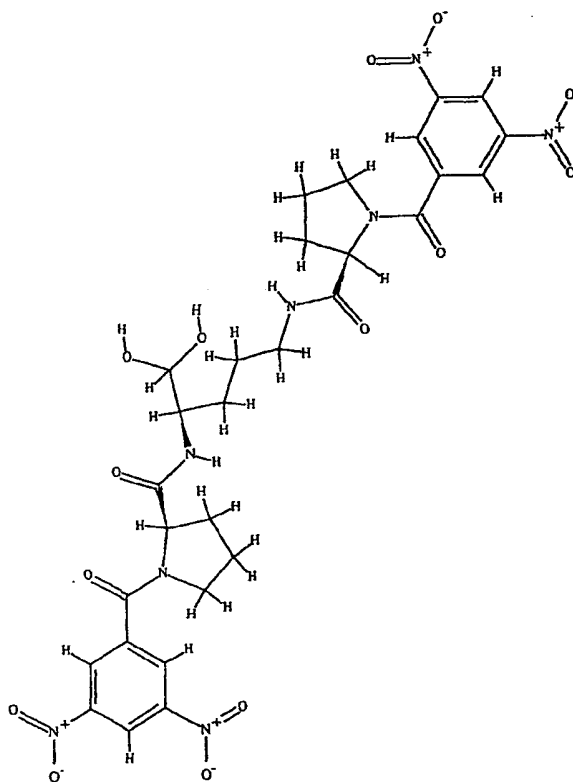


SUBSTITUTE SHEET (RULE 26)

399

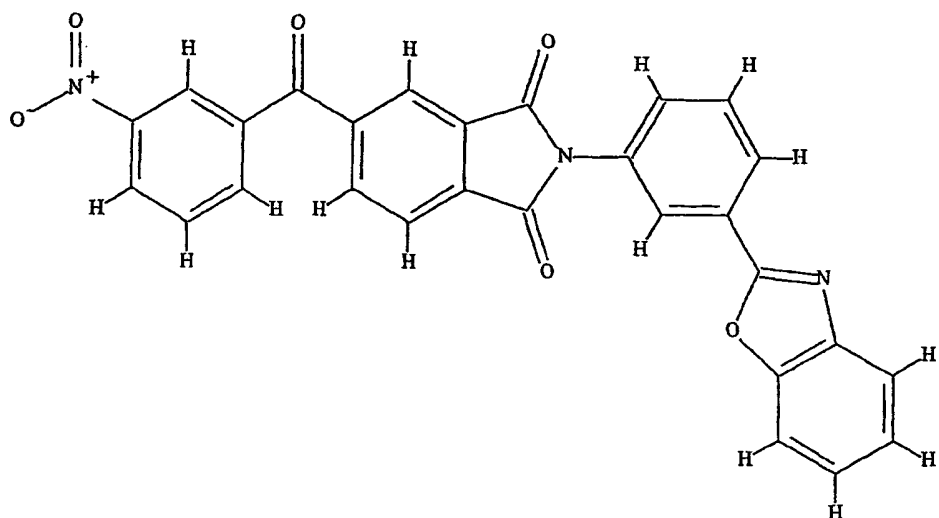


400

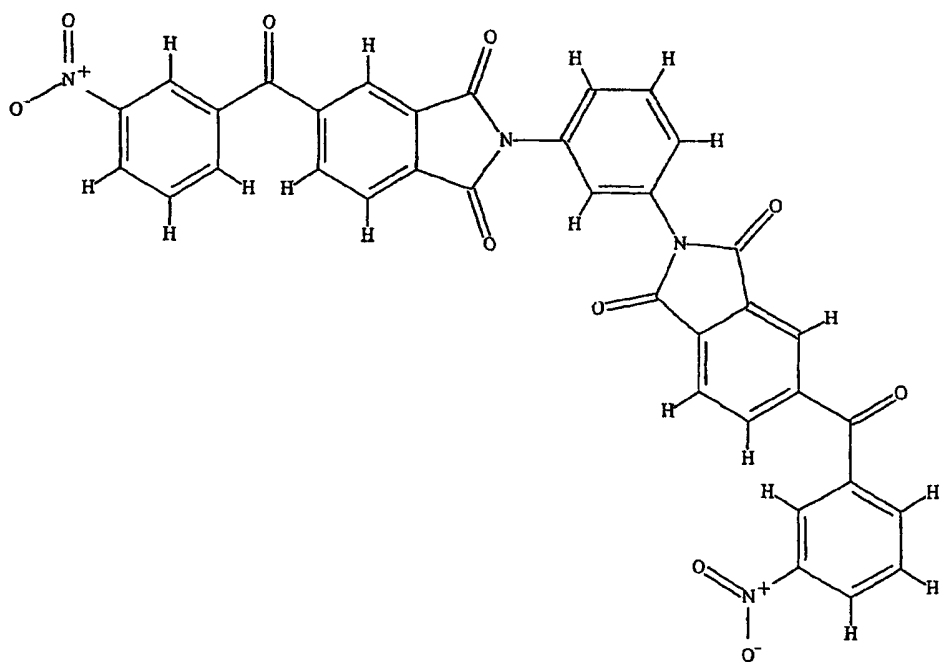


SUBSTITUTE SHEET (RULE 26)

401

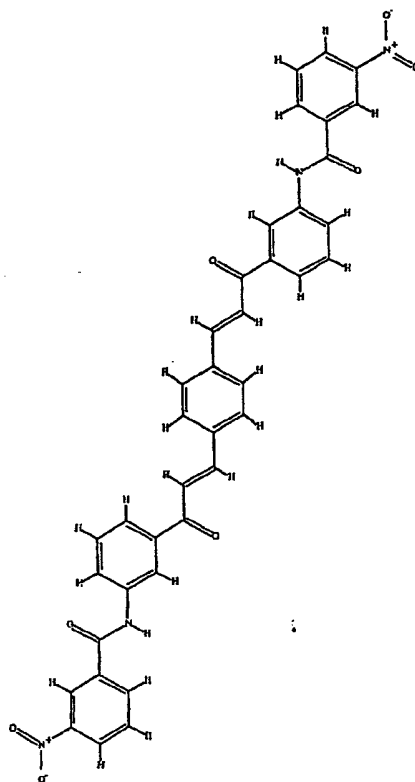


402

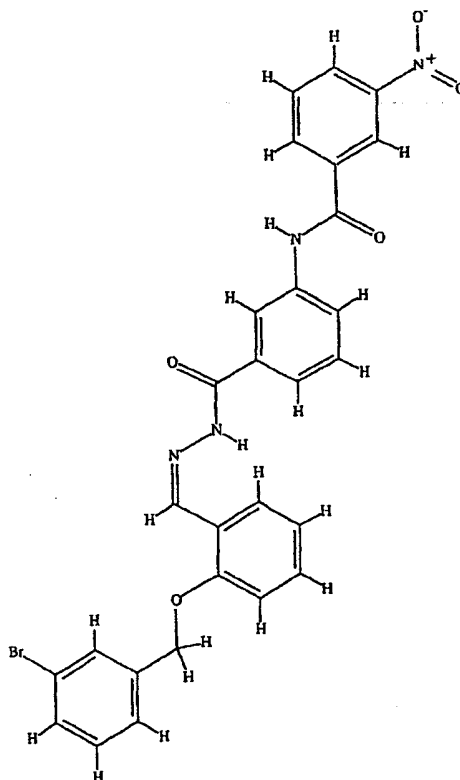


202/248

403



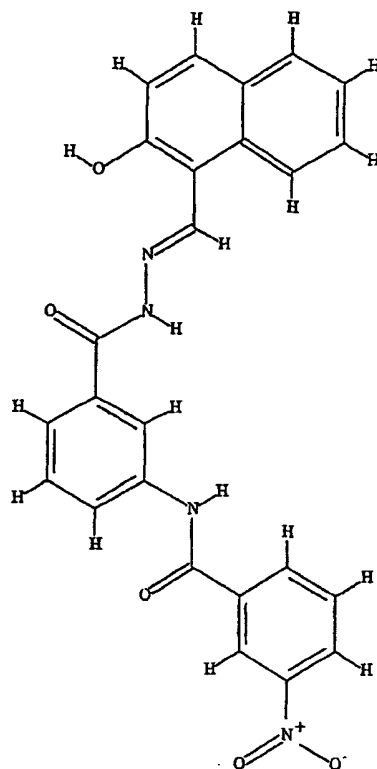
404



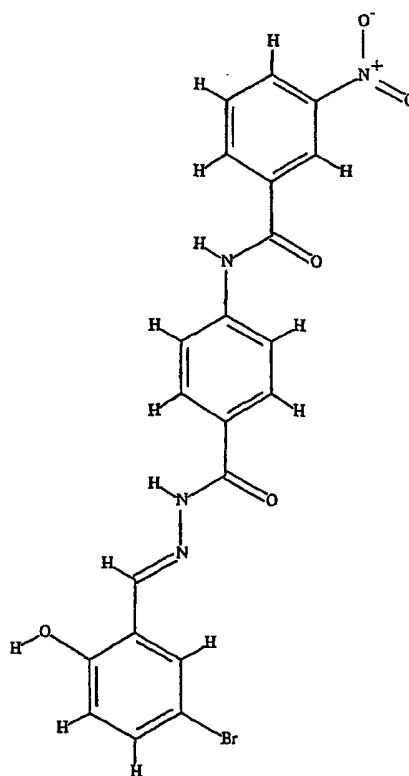
SUBSTITUTE SHEET (RULE 26)

203/248

405



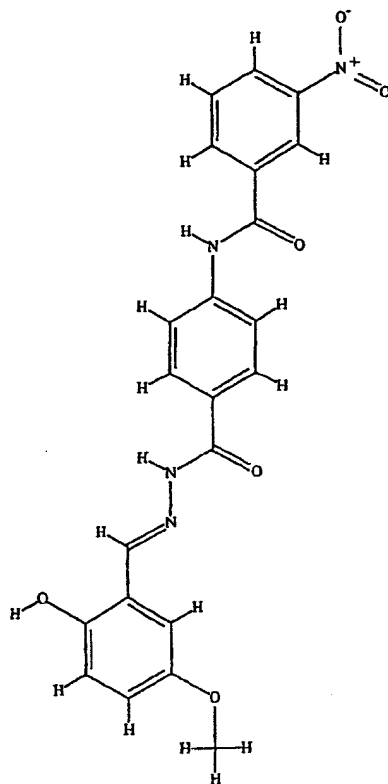
406



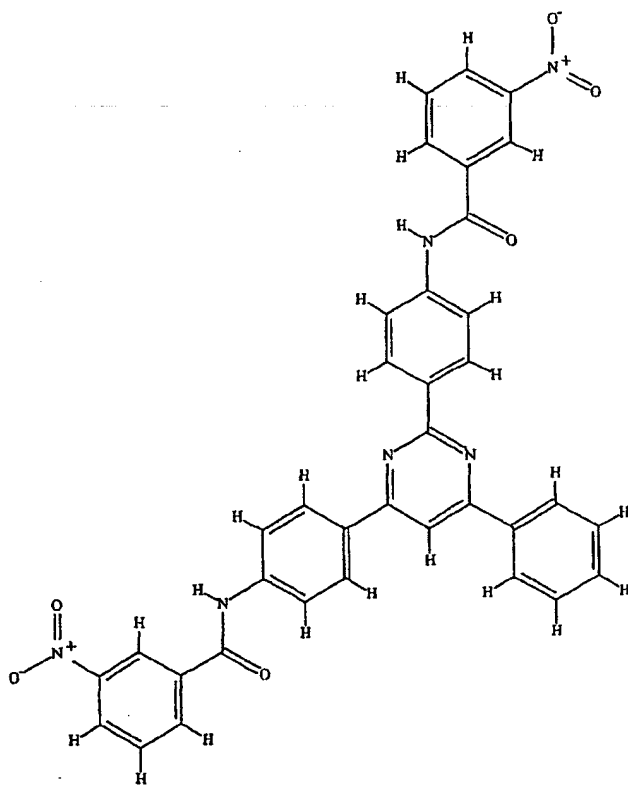
SUBSTITUTE SHEET (RULE 26)

204/248

407

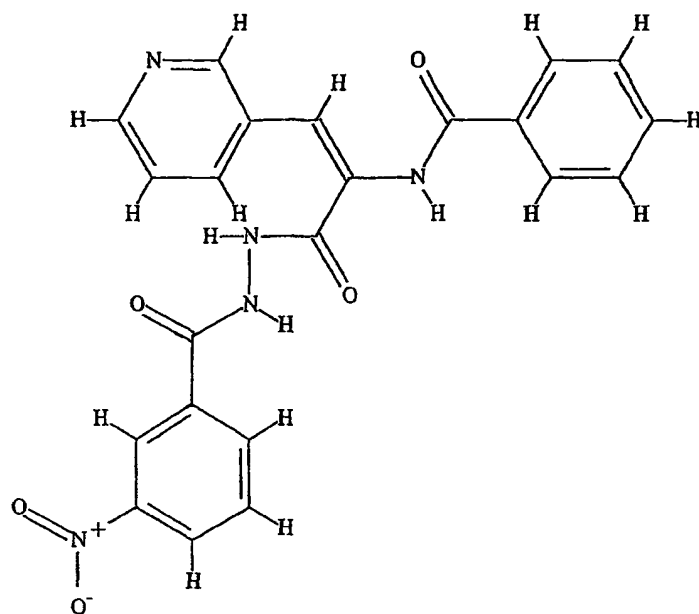


408

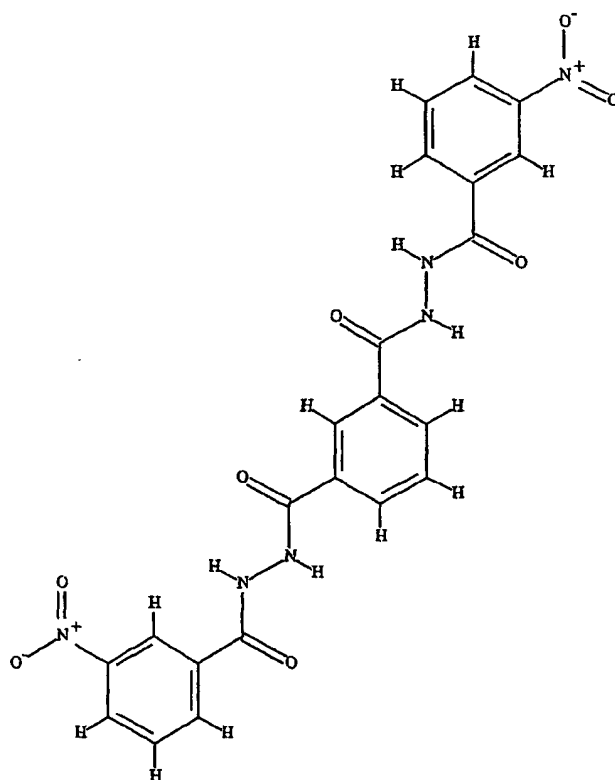


SUBSTITUTE SHEET (RULE 26)

409

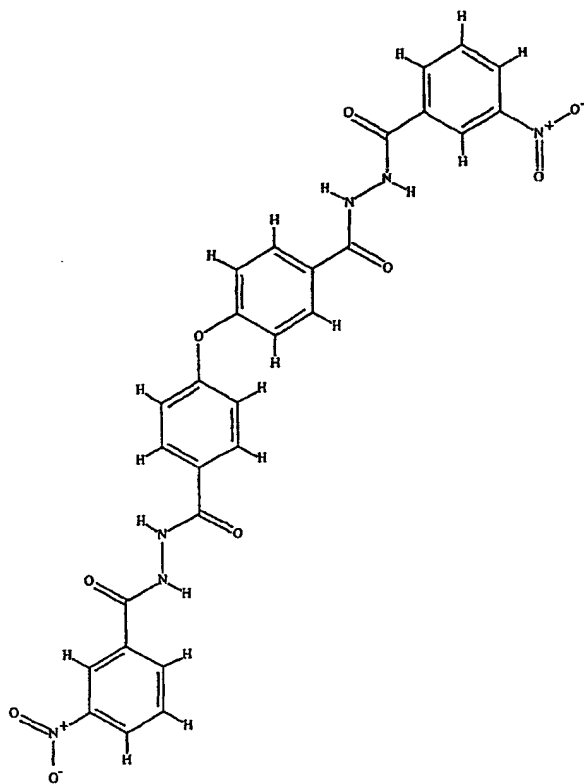


410

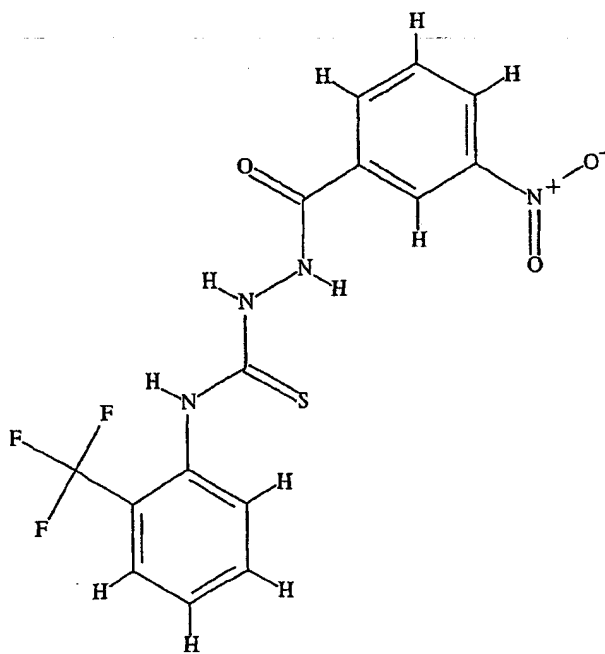




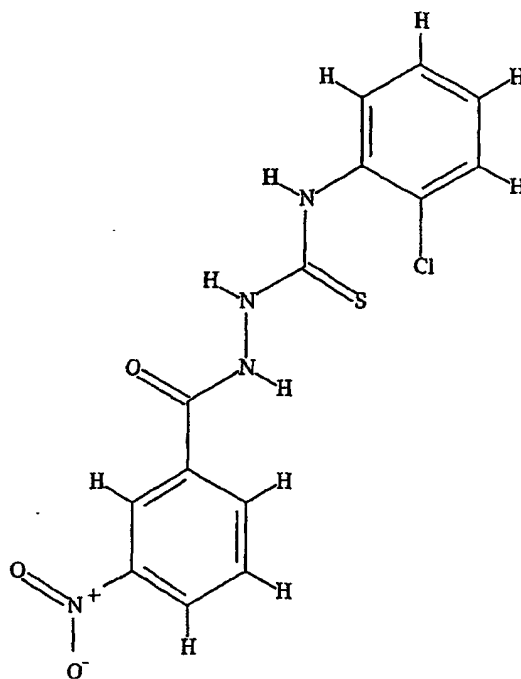
411



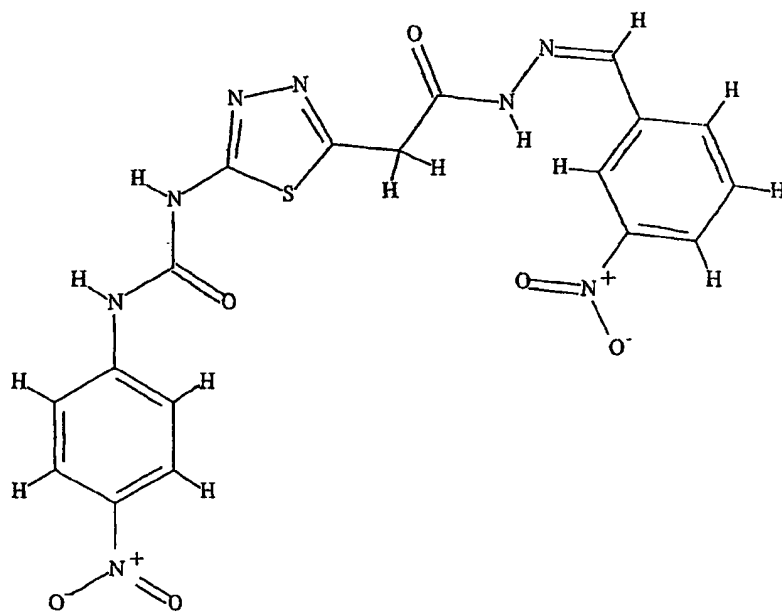
412



413

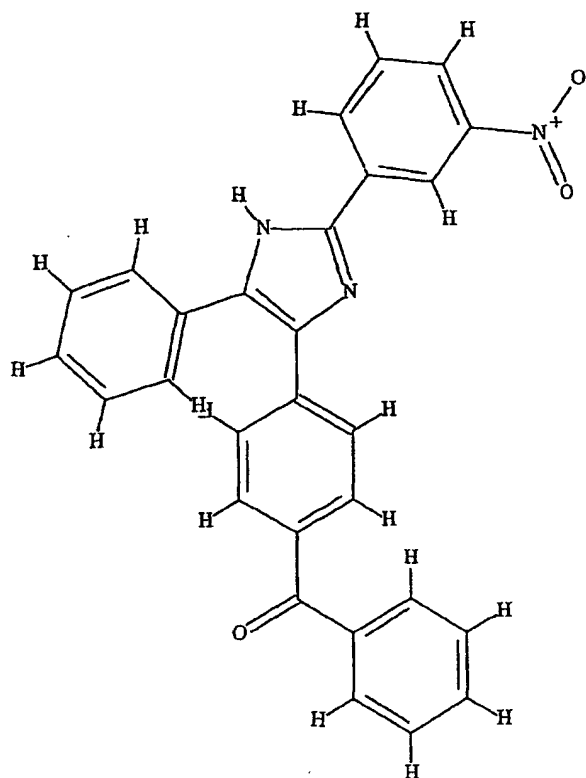


414

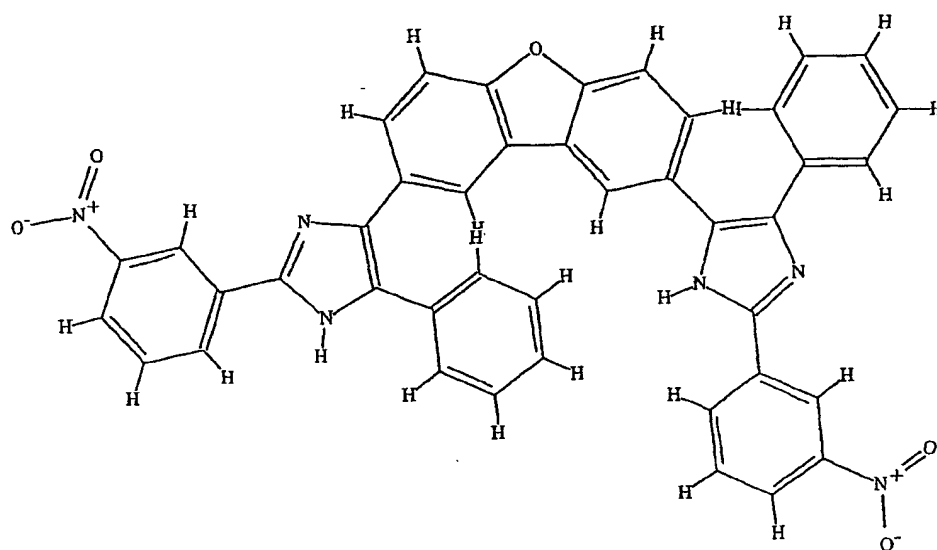


SUBSTITUTE SHEET (RULE 26)

415

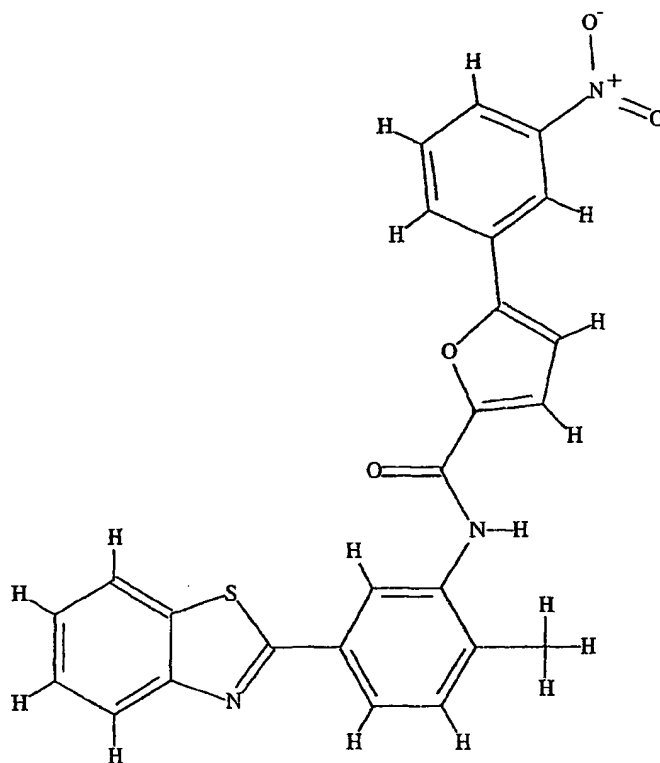


416

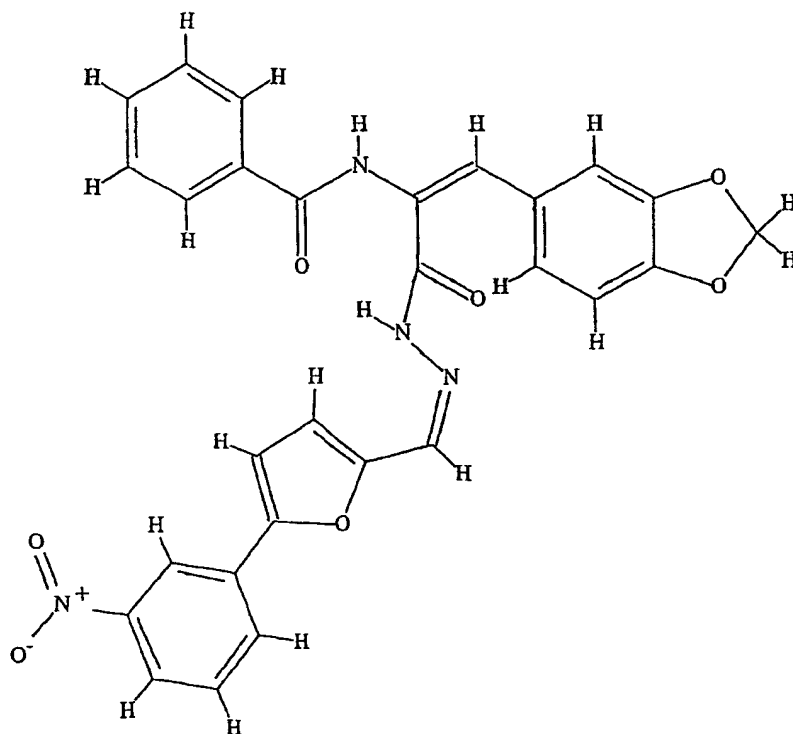


SUBSTITUTE SHEET (RULE 26)

417

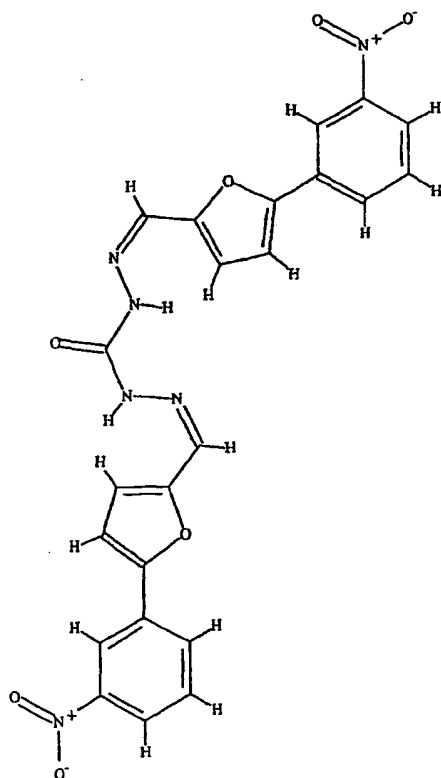


418

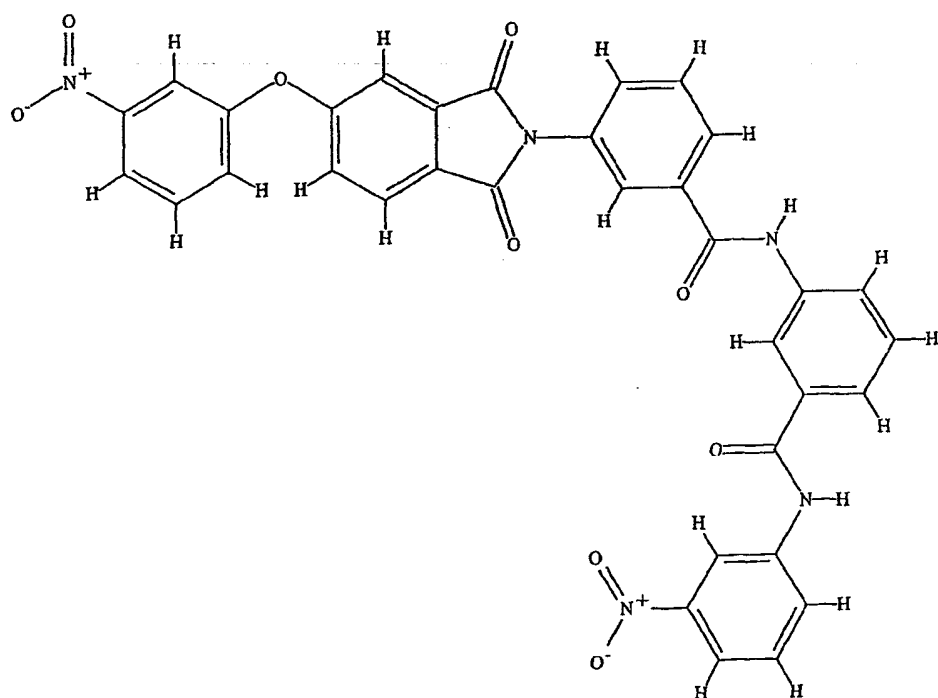


SUBSTITUTE SHEET (RULE 26)

419

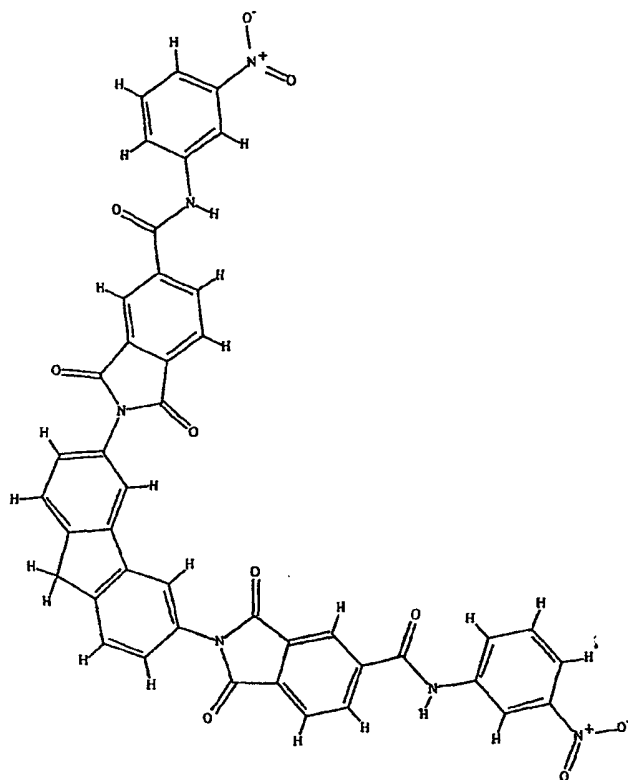


420

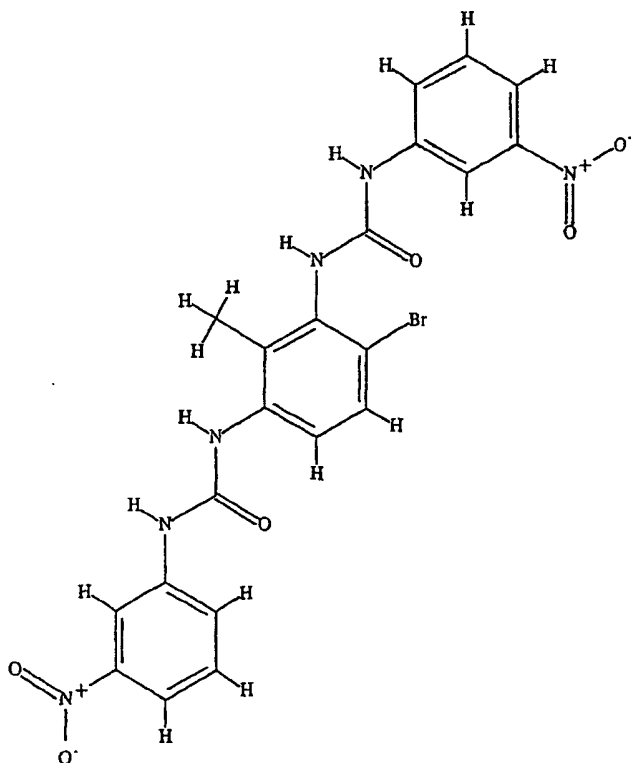


211/248

421

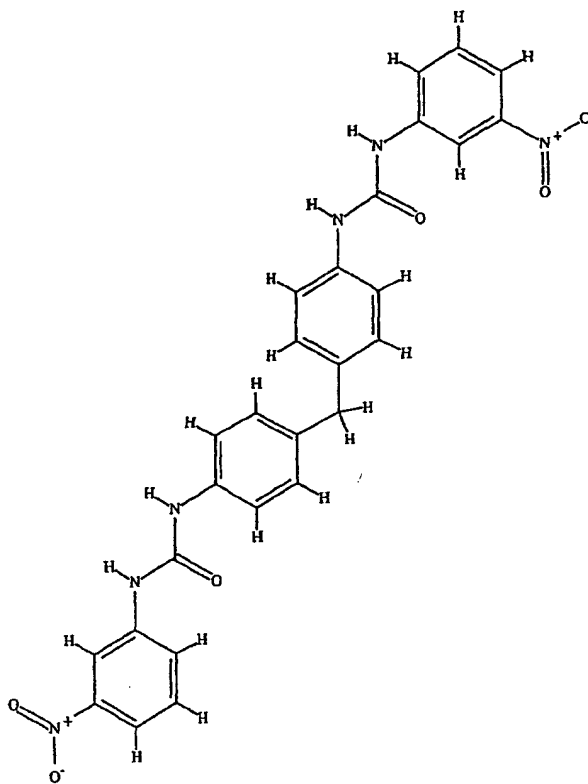


422

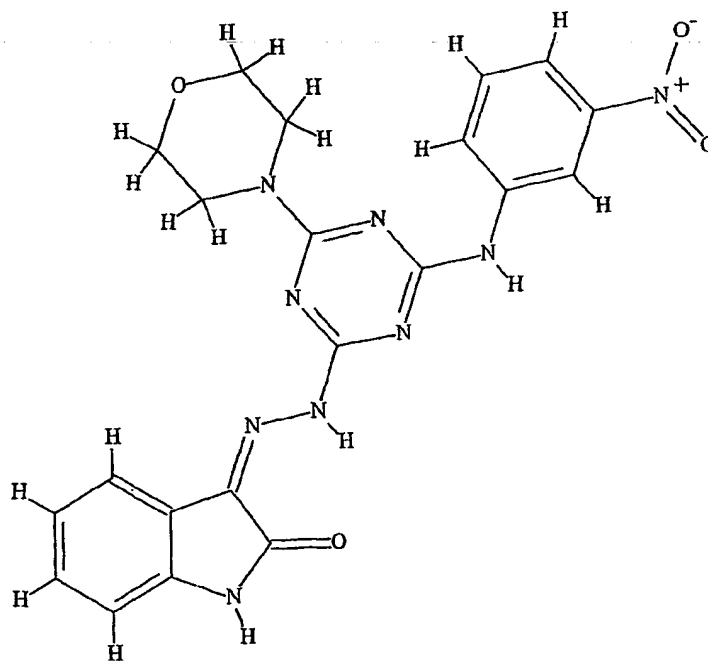


SUBSTITUTE SHEET (RULE 26)

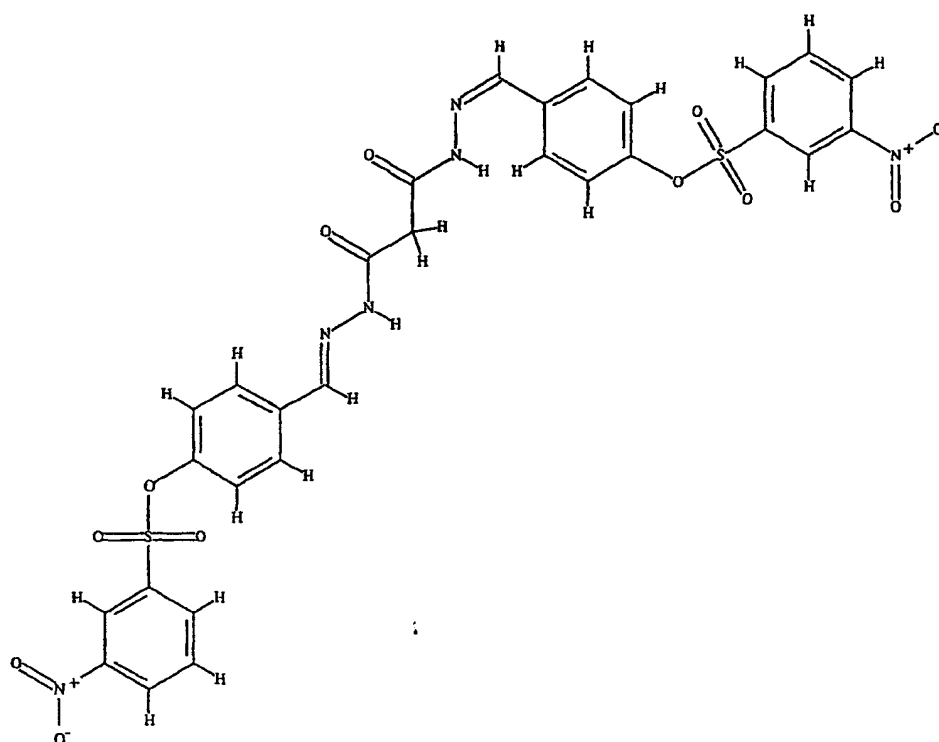
423



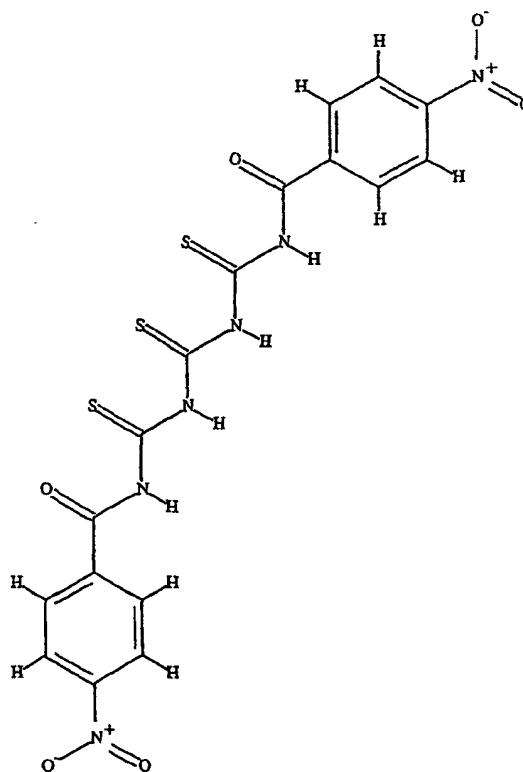
424



425



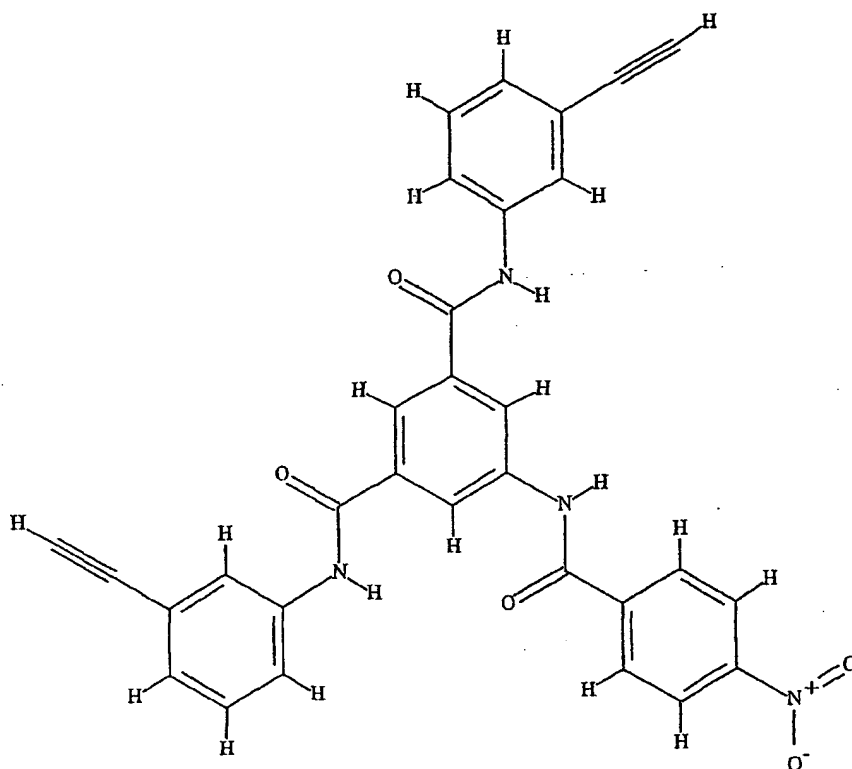
426



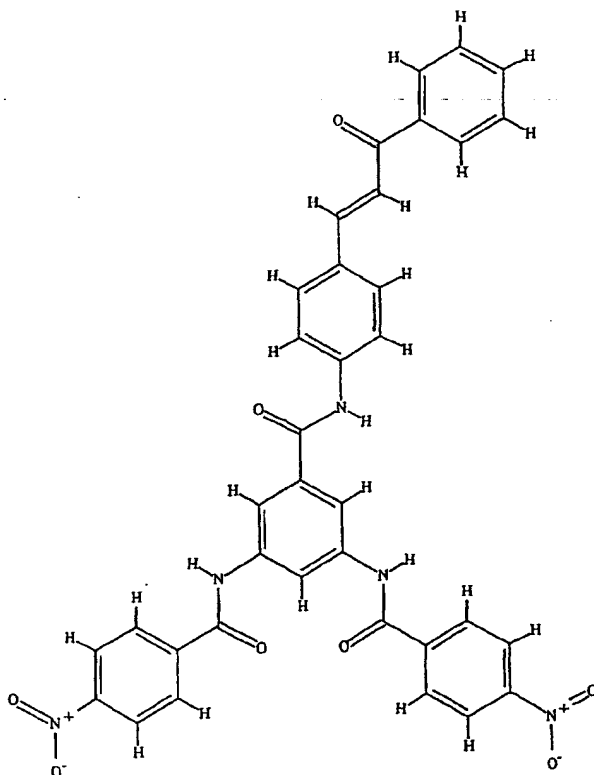
SUBSTITUTE SHEET (RULE 26)



427



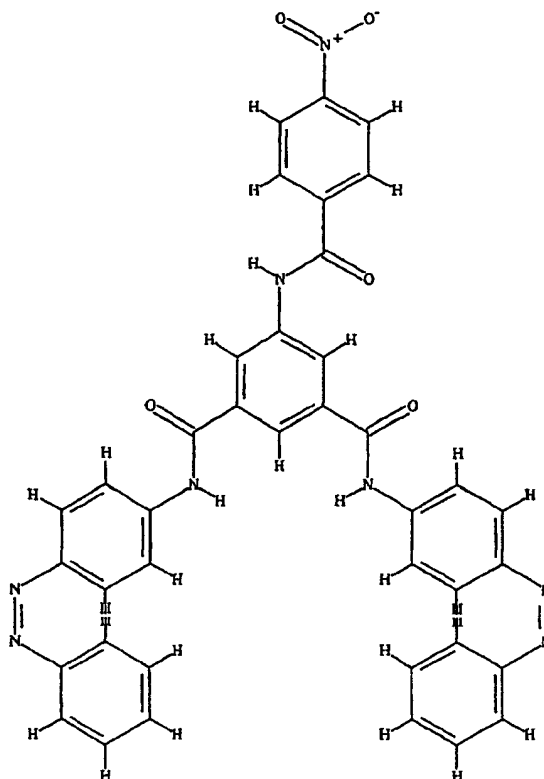
428



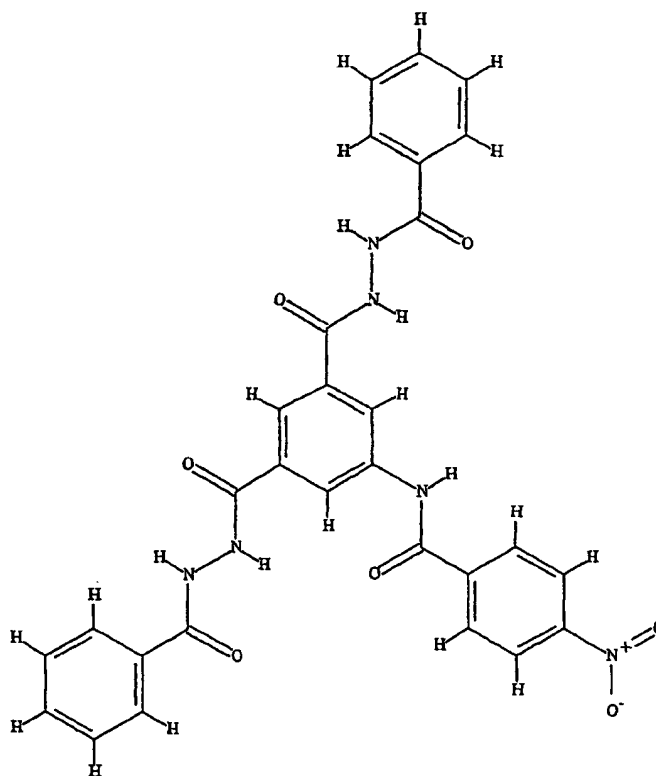
SUBSTITUTE SHEET (RULE 26)

215/248

429

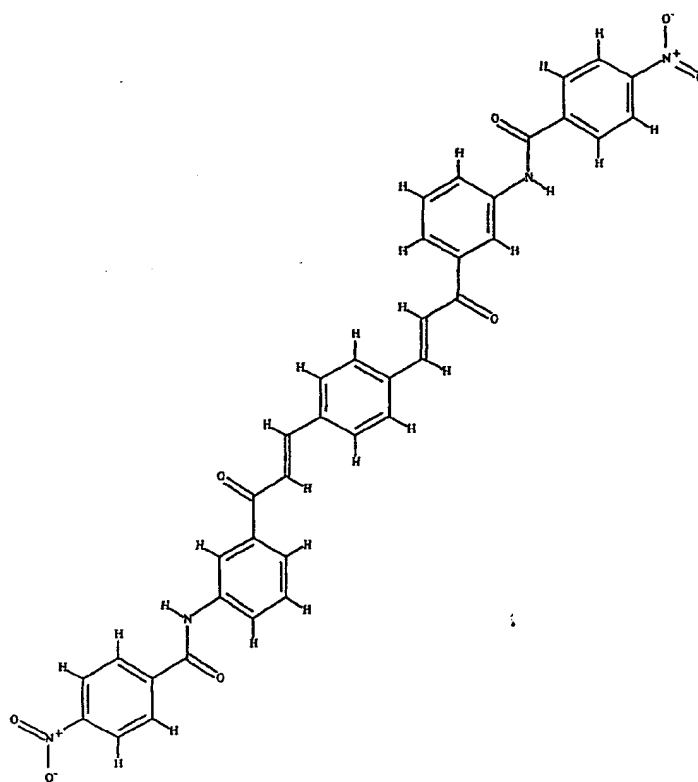


430

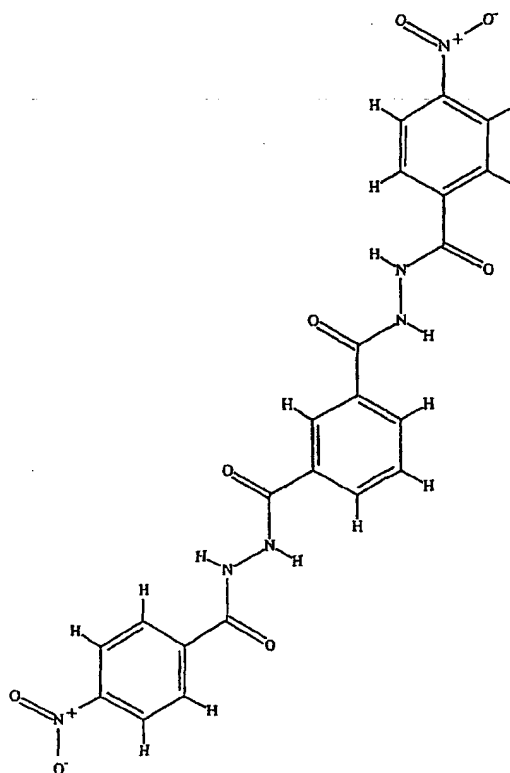


SUBSTITUTE SHEET (RULE 26)

431



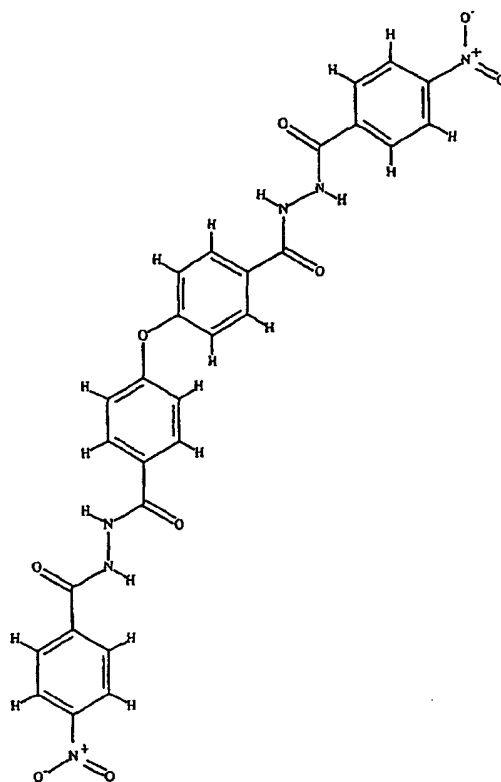
432



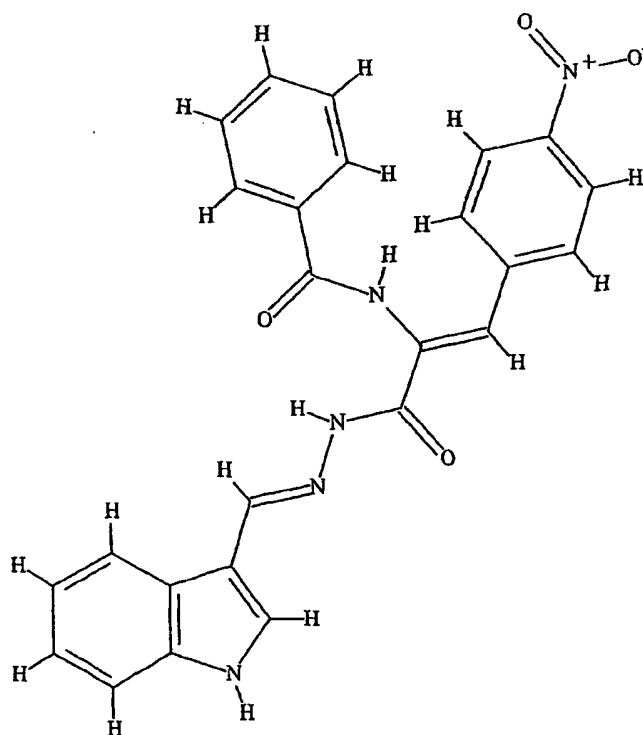
SUBSTITUTE SHEET (RULE 26)

217/248

433

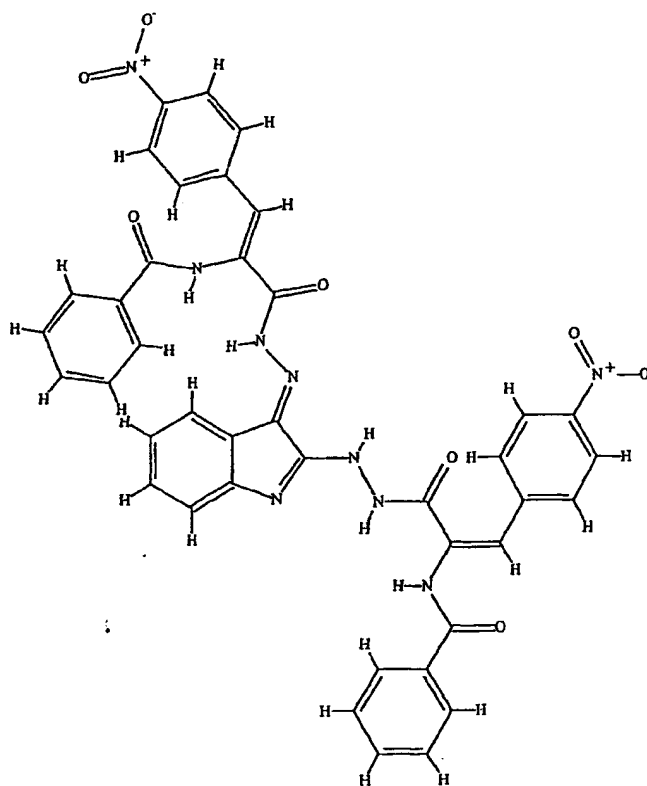


434

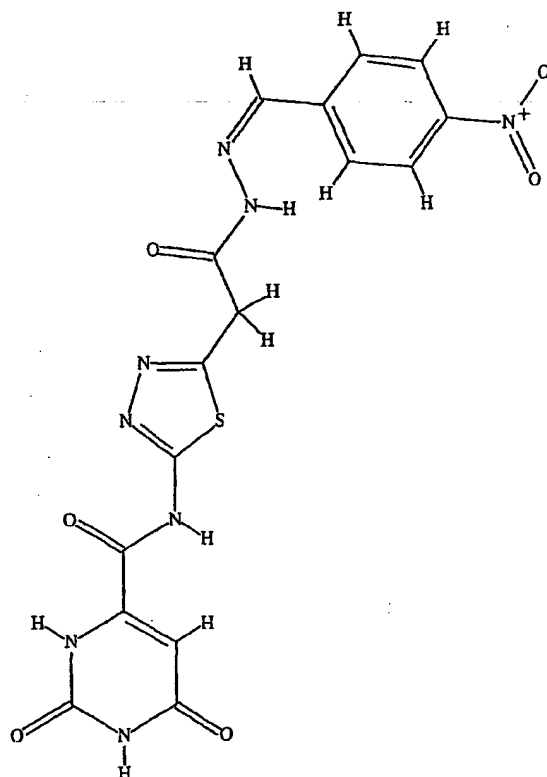


SUBSTITUTE SHEET (RULE 26)

435



436



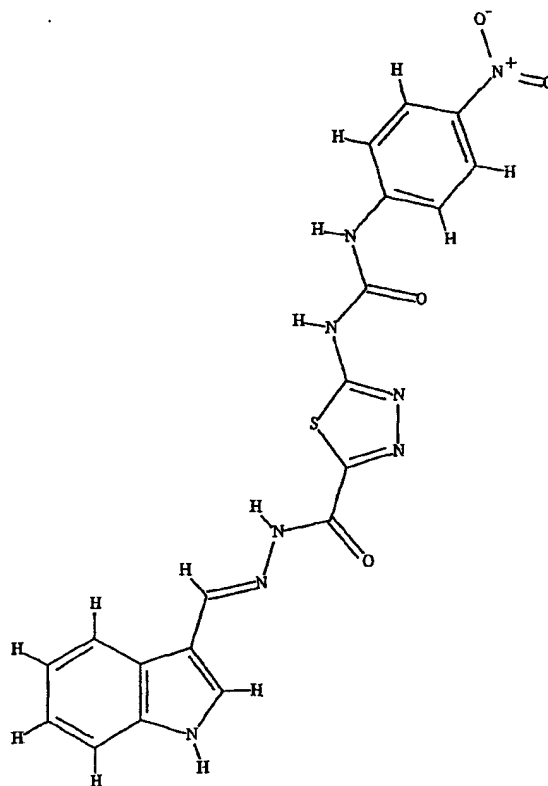
**SUBSTITUTE SHEET (RULE 26)**

O=[N+]([O-])c1ccc2c(c1)c3c(ncn3C(=O)Nc4c[nH]c5c4C(=O)Nc6c[nH]c7cc([N+]([O-])=O)ccc67)c2

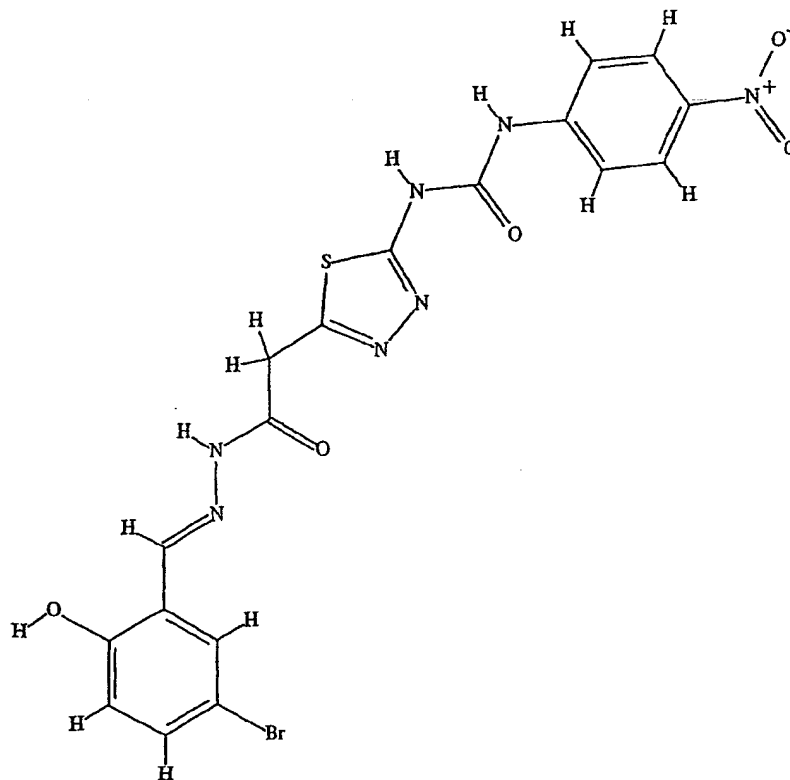
The chemical structure shows a central 1,3-bis(4-methylphenyl)-5-((4-methylphenyl)diazenyl)-4,5-dihydro-1H-pyrazol-4-one molecule. It consists of a central pyrazole ring substituted with two 4-methylphenyl groups and a 4-methylphenyl diazenyl group. The pyrazole ring is in a 4,5-dihydro state, and the diazenyl group is in a trans configuration. The 4-methylphenyl groups are represented by benzene rings with a methyl group (CH<sub>3</sub>) at the para position.

**SUBSTITUTE SHEET (RULE 26)**

439

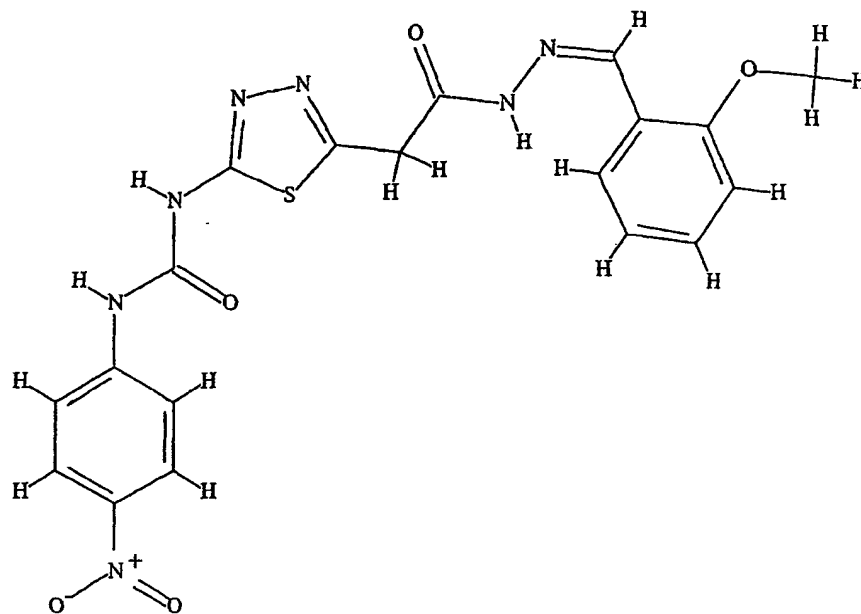


440

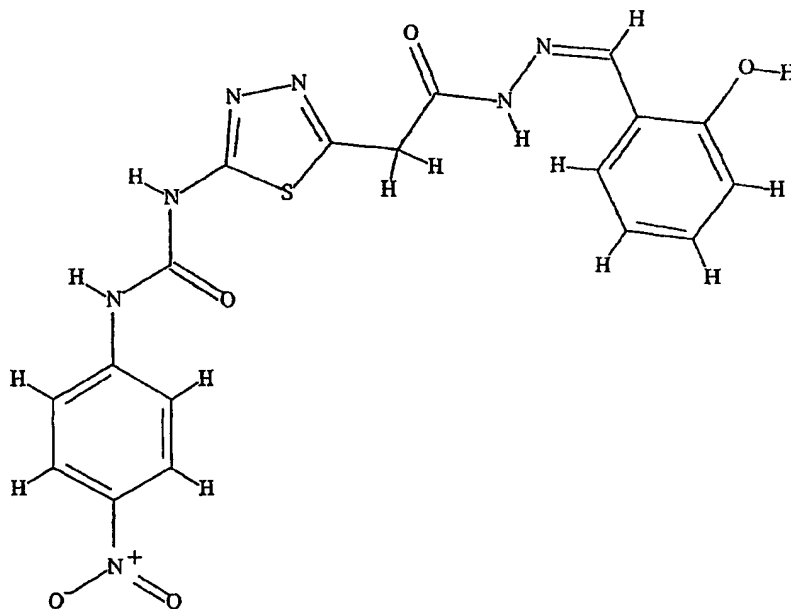


SUBSTITUTE SHEET (RULE 26)

441



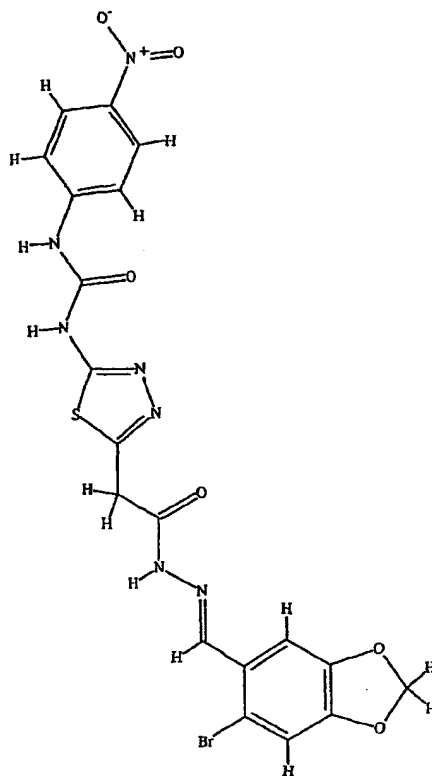
442



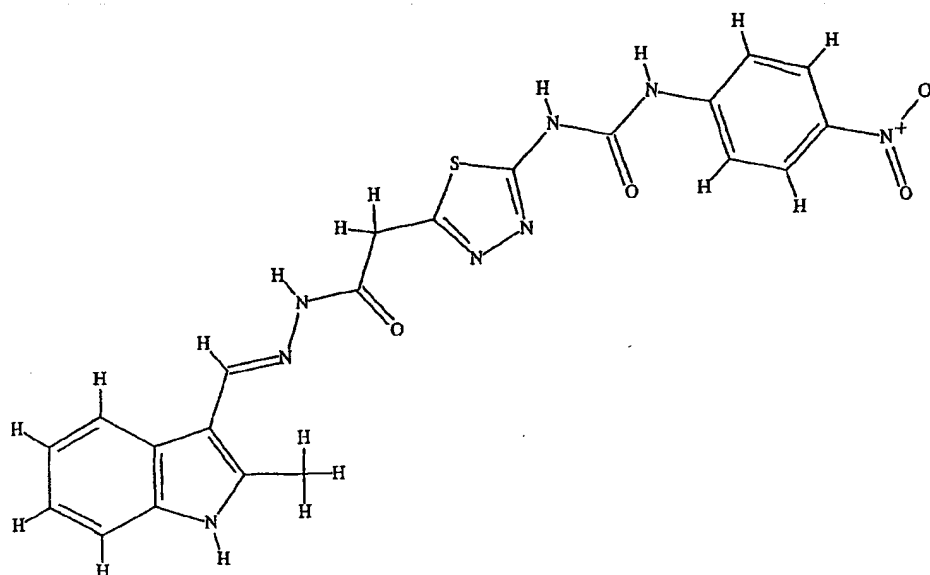


222/248

443

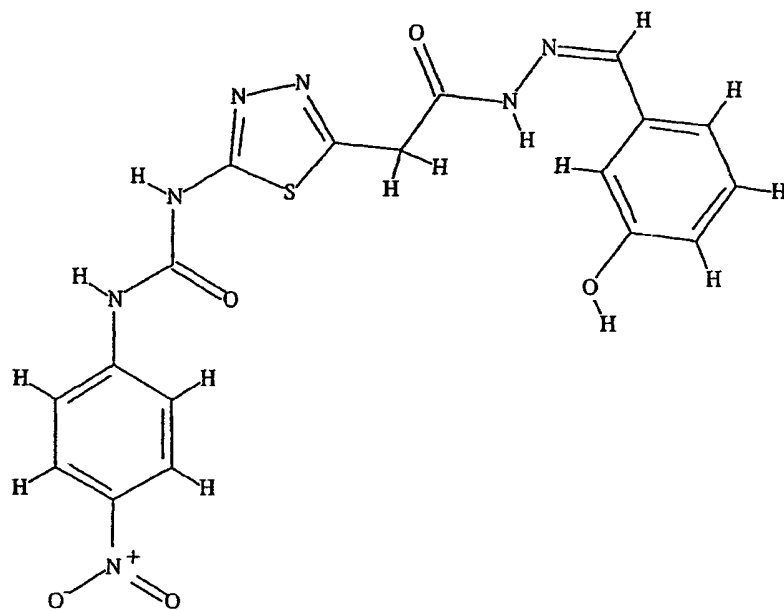


444

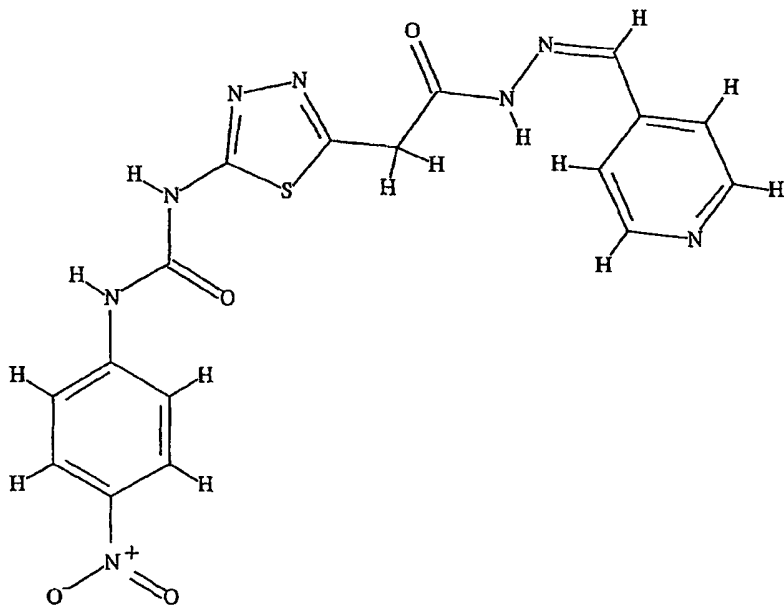


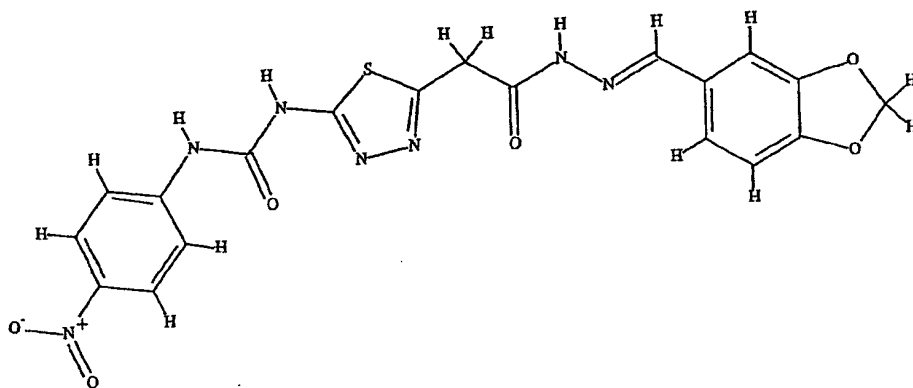
SUBSTITUTE SHEET (RULE 26)

445

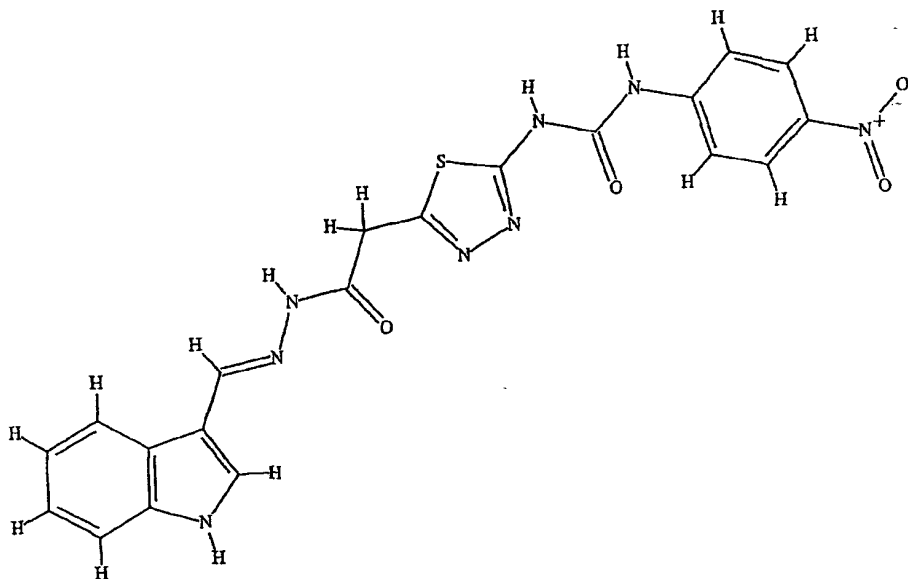


446



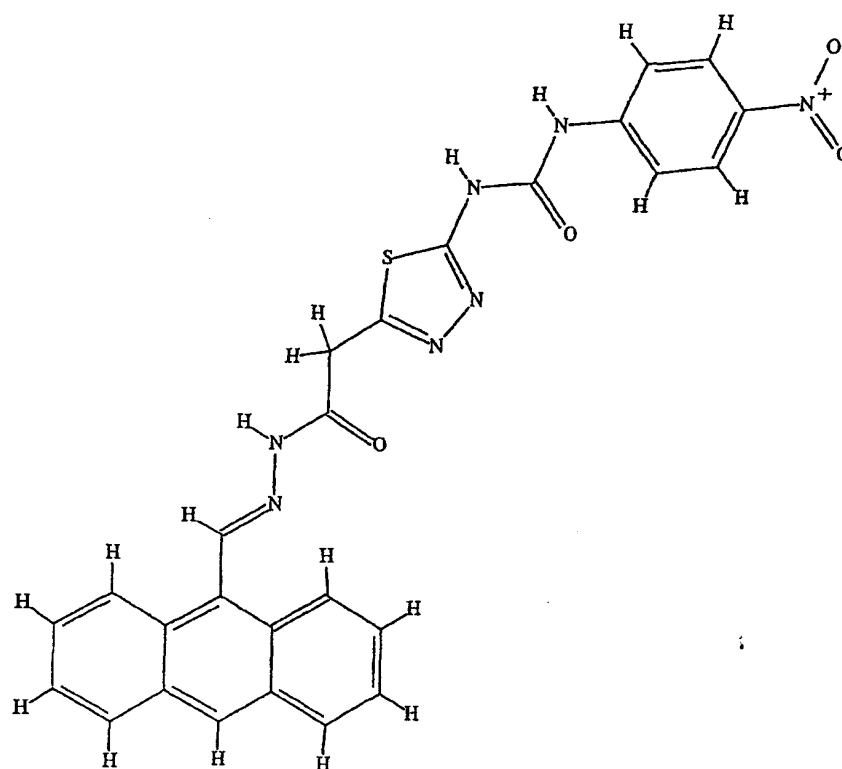


448

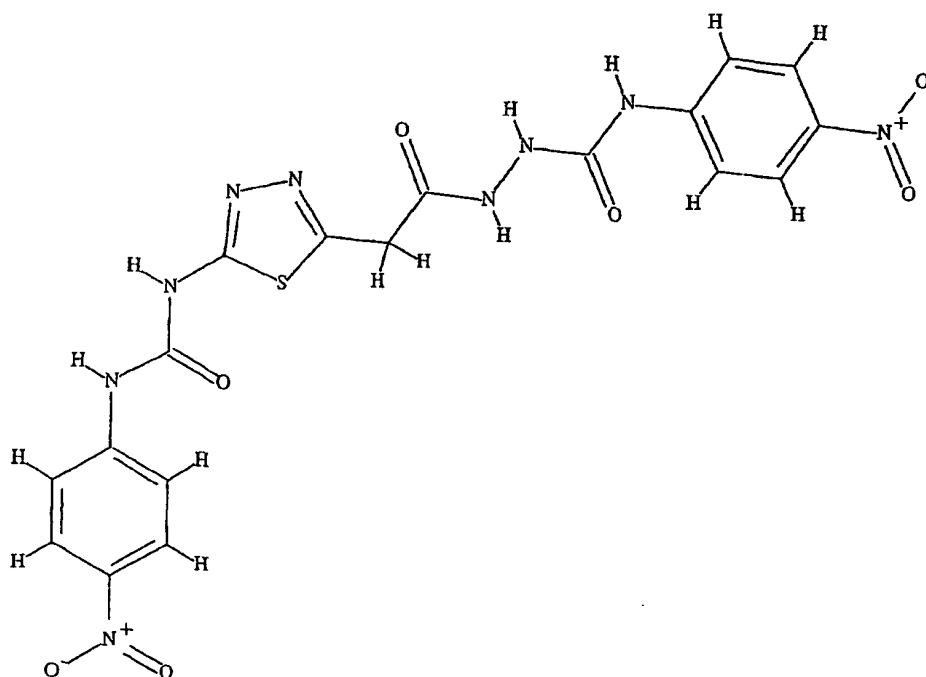


BNSDOCID: <WO\_\_\_\_\_0168122A2\_1\_>

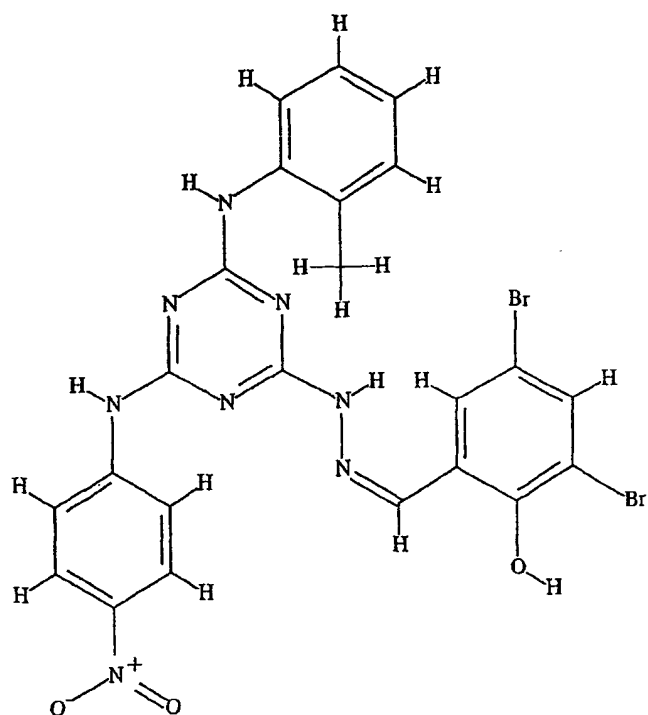
449



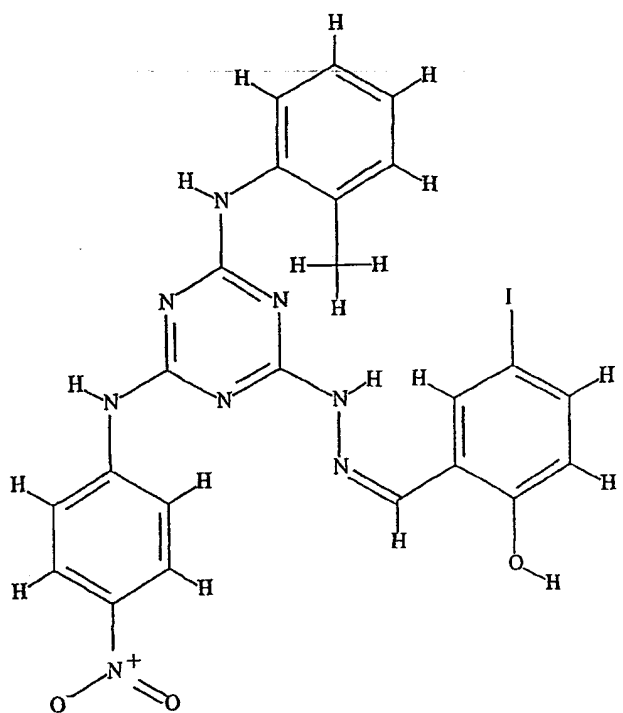
450



451

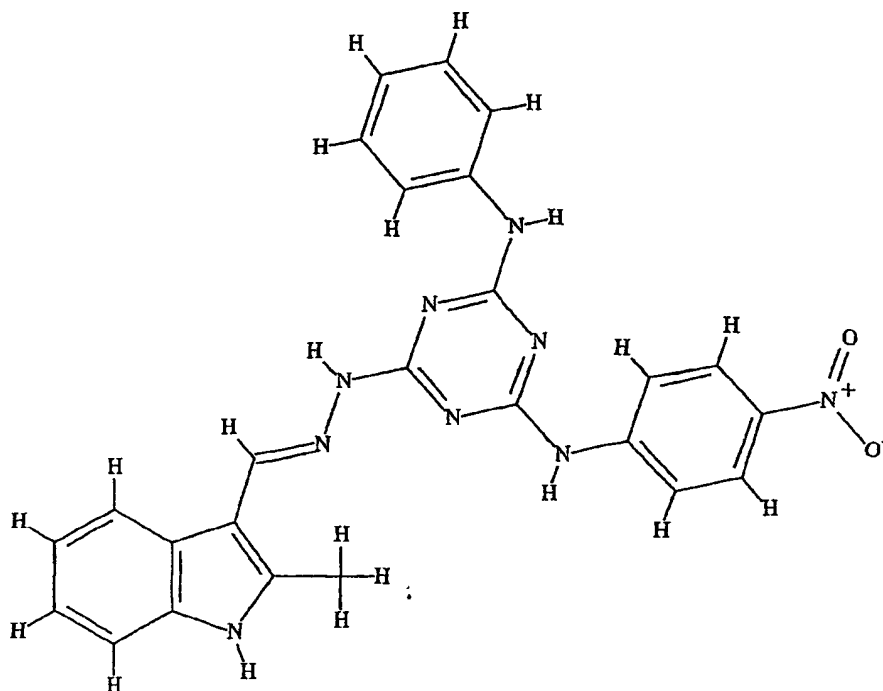


452

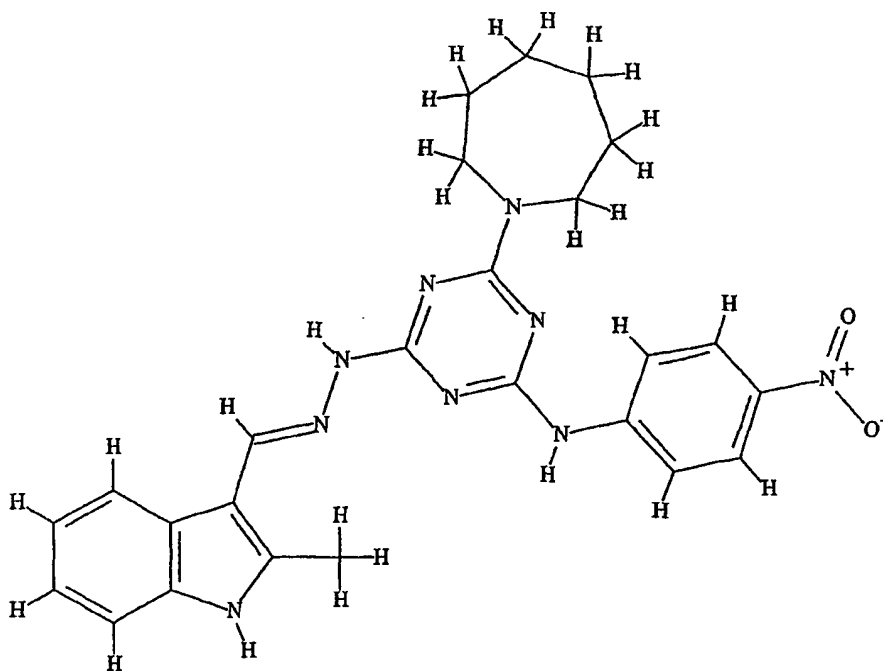


SUBSTITUTE SHEET (RULE 26)

453

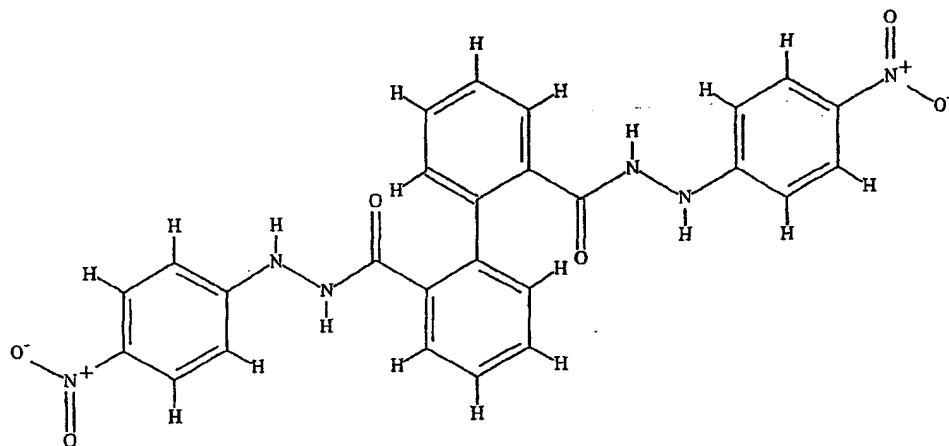


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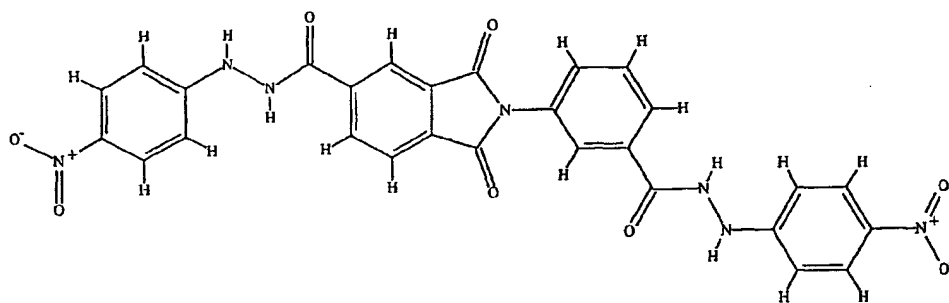


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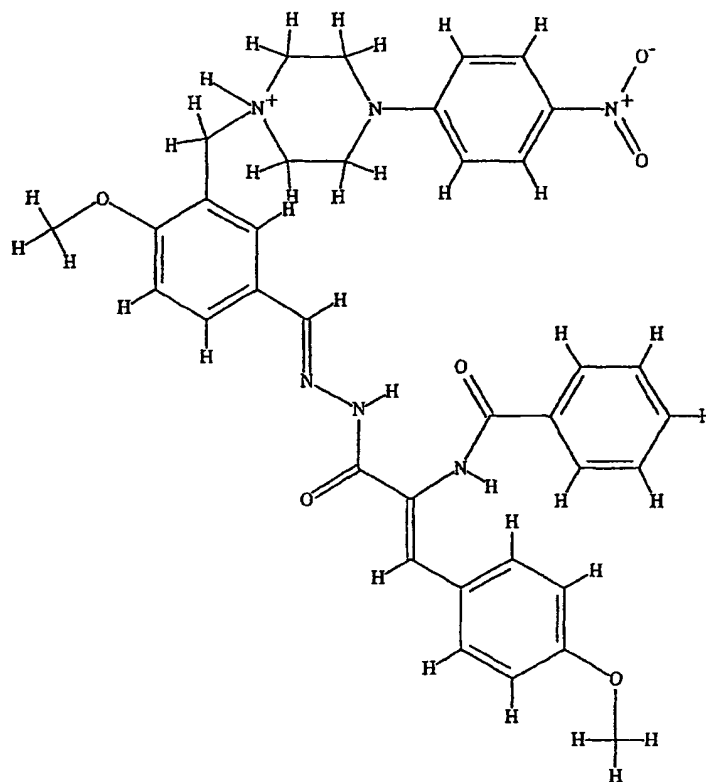


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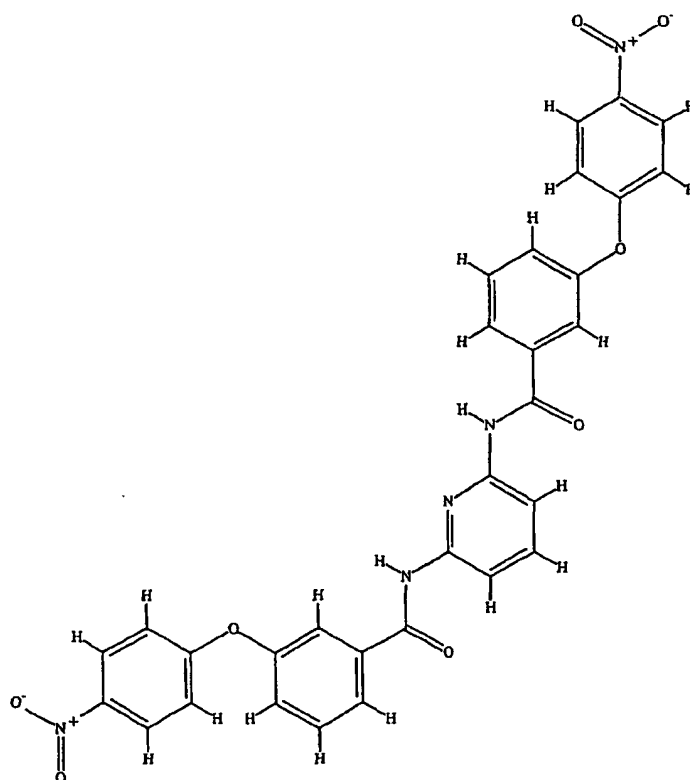


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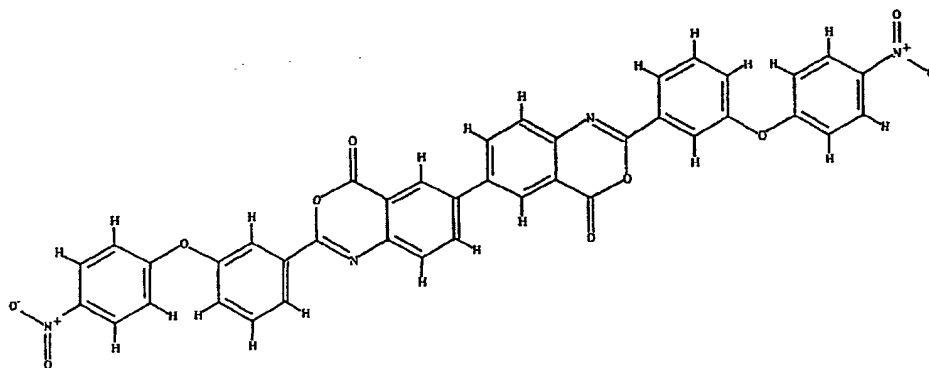
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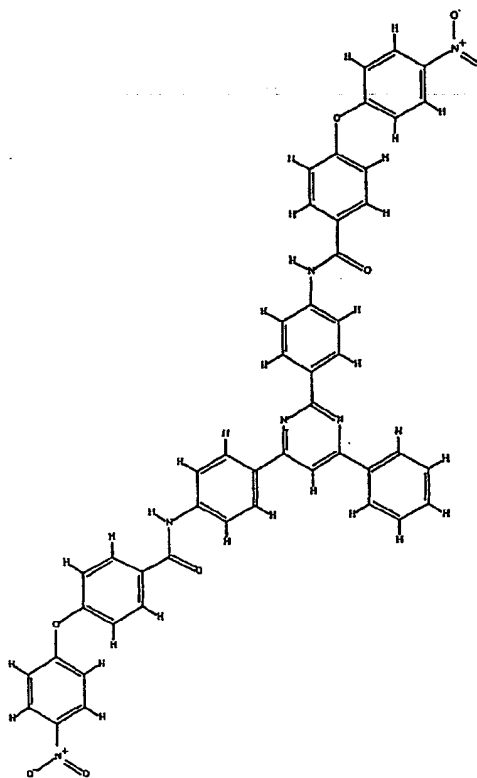
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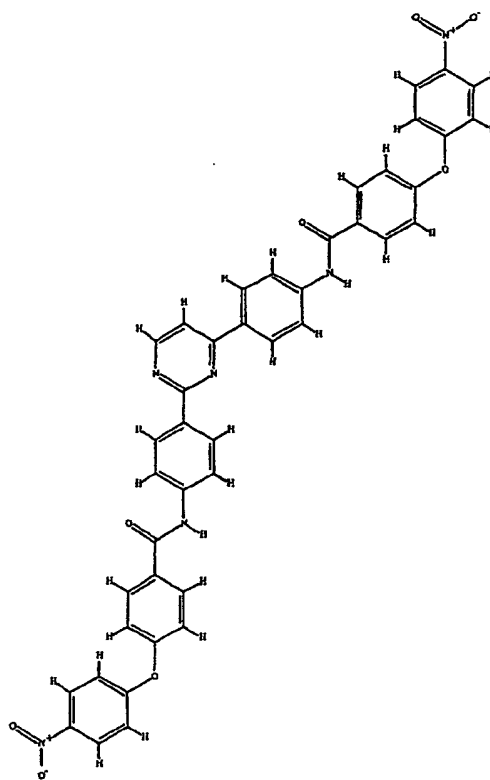


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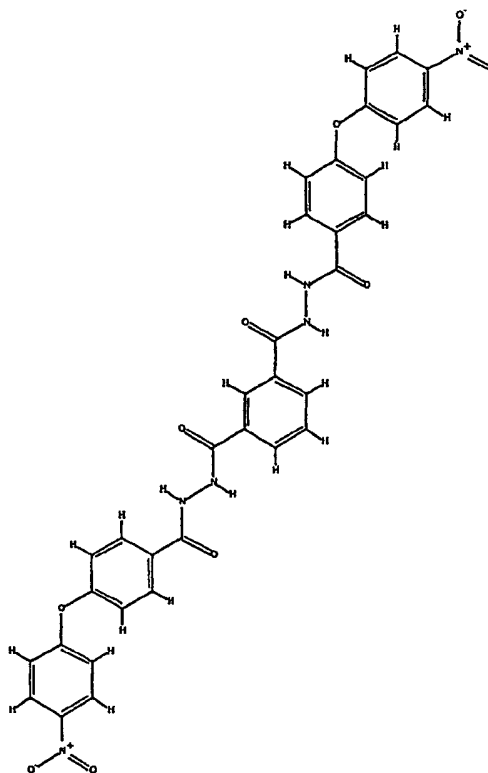


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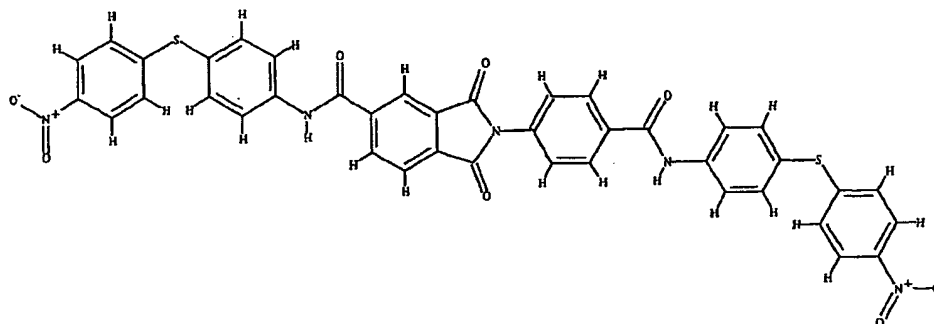


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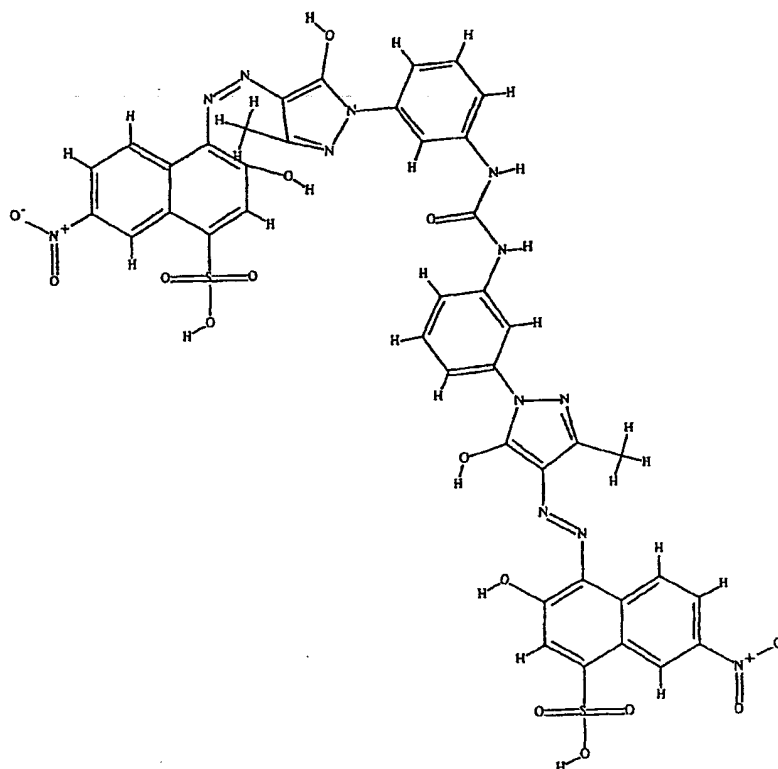


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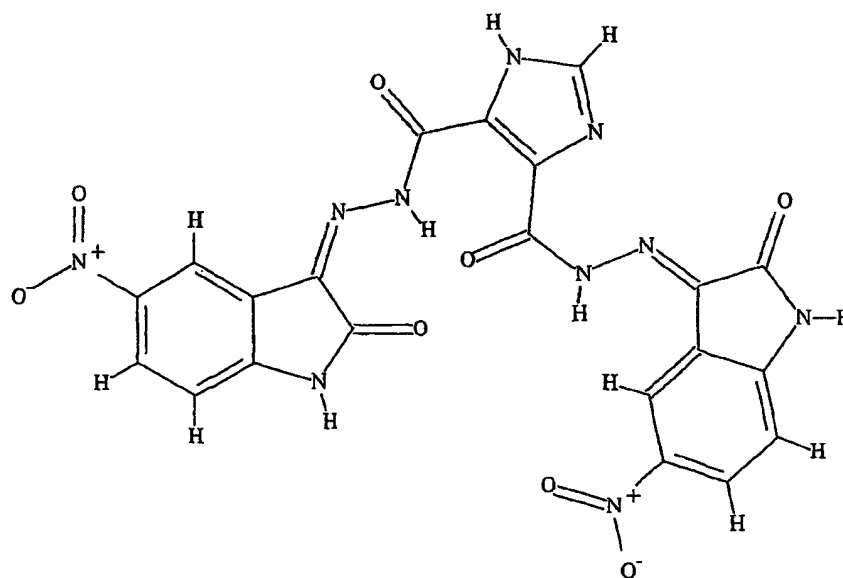
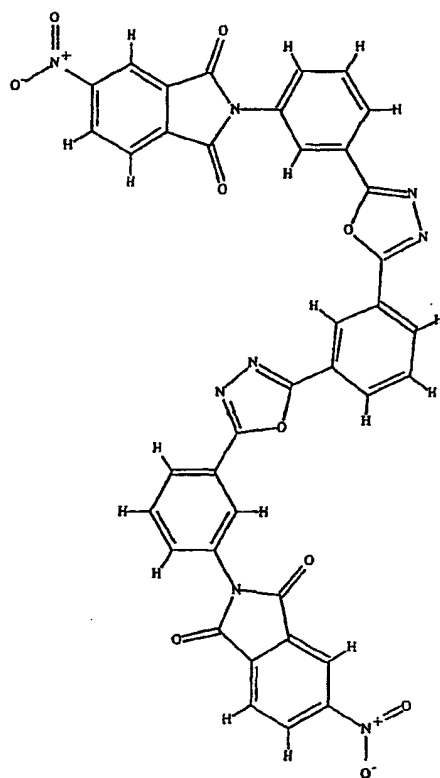
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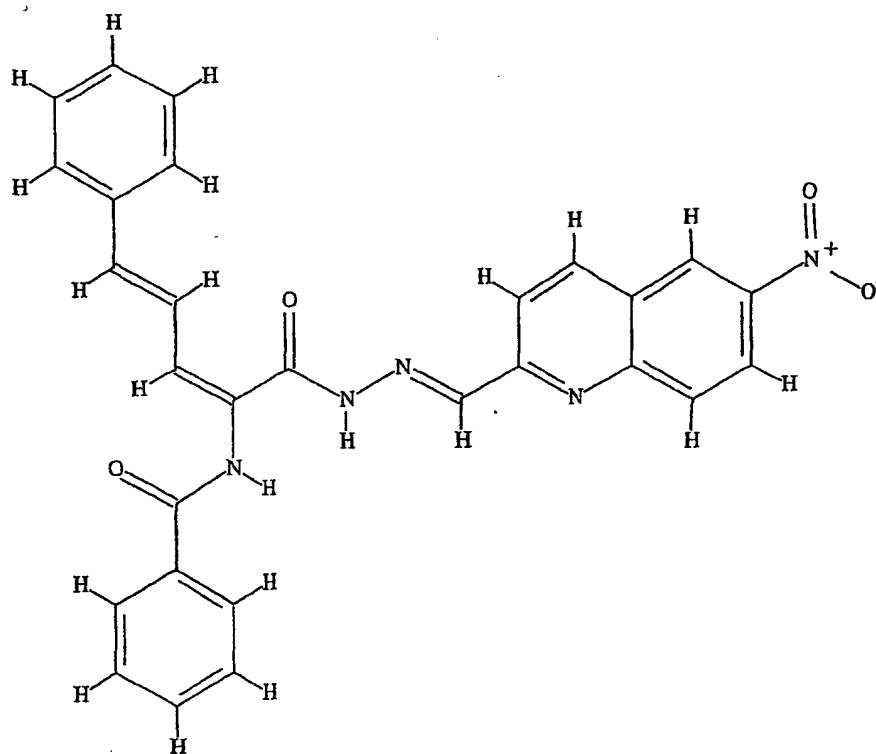
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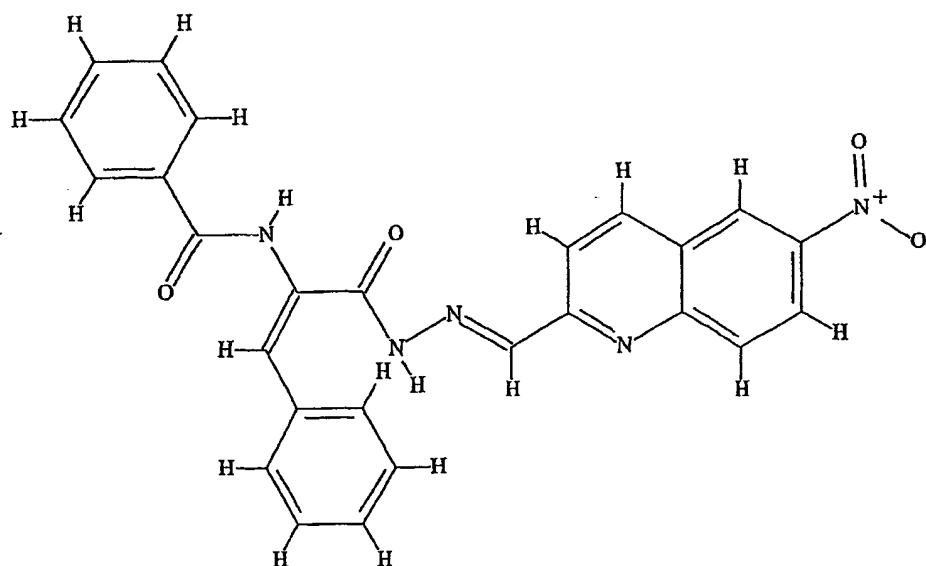
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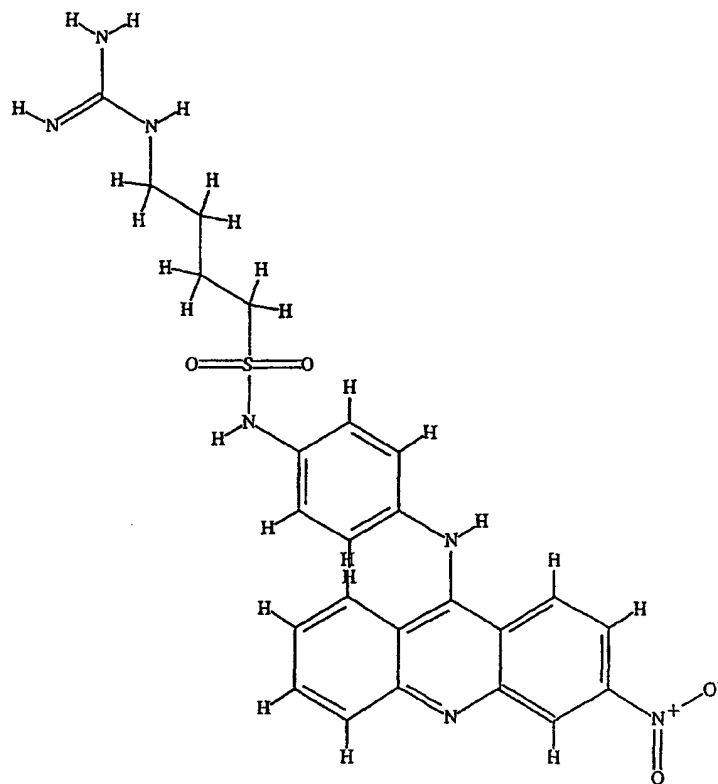


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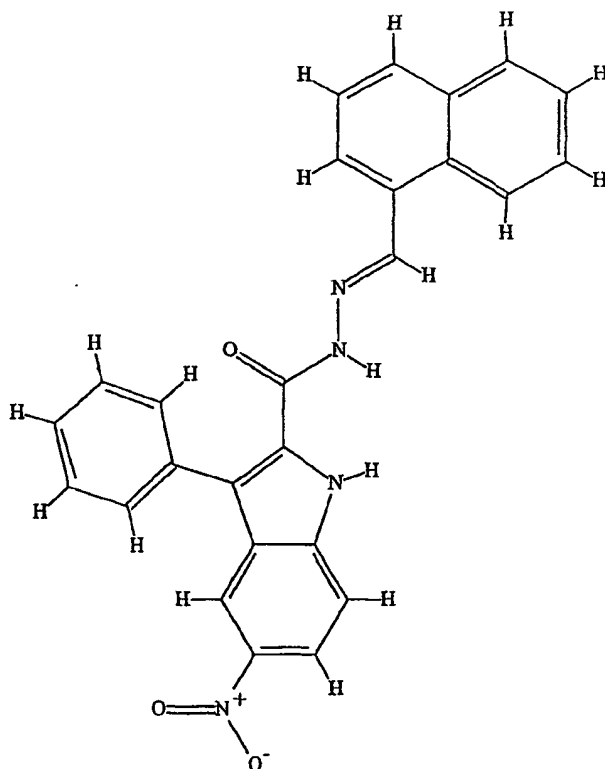


235/248

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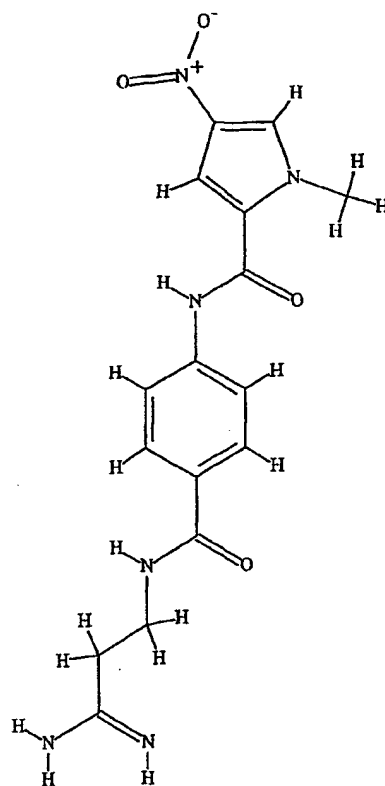
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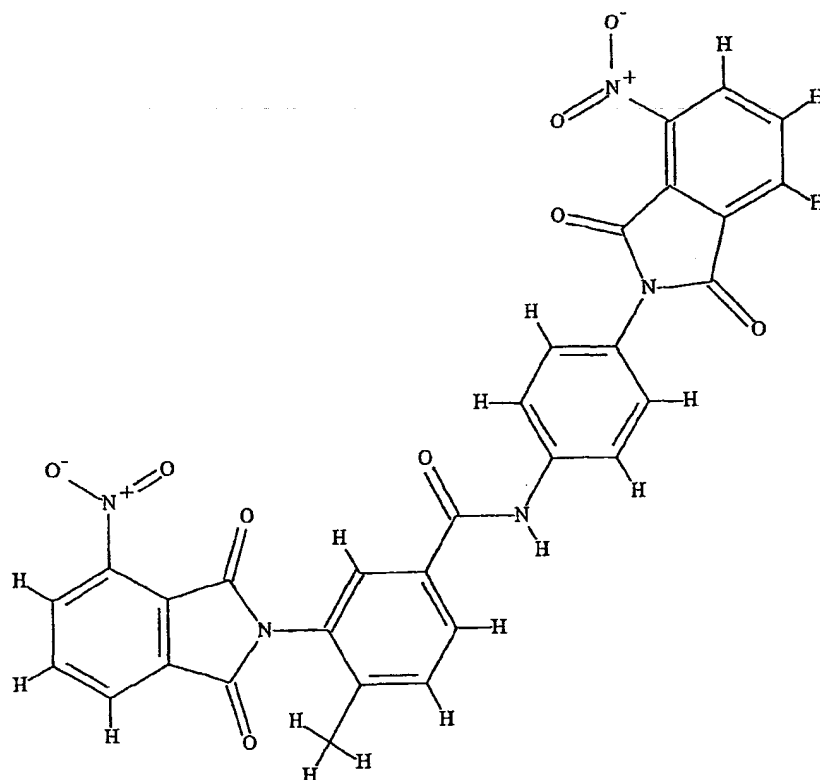
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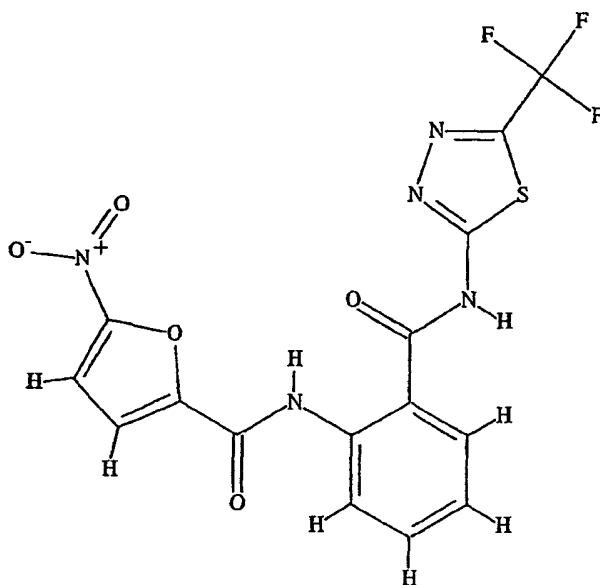


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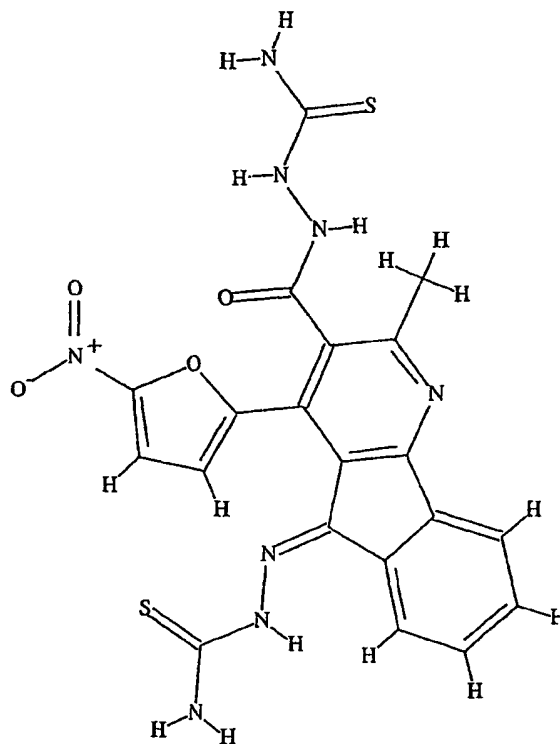


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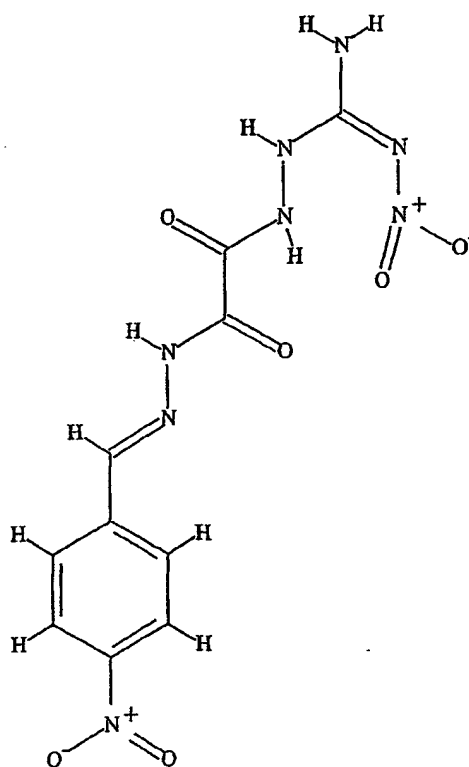
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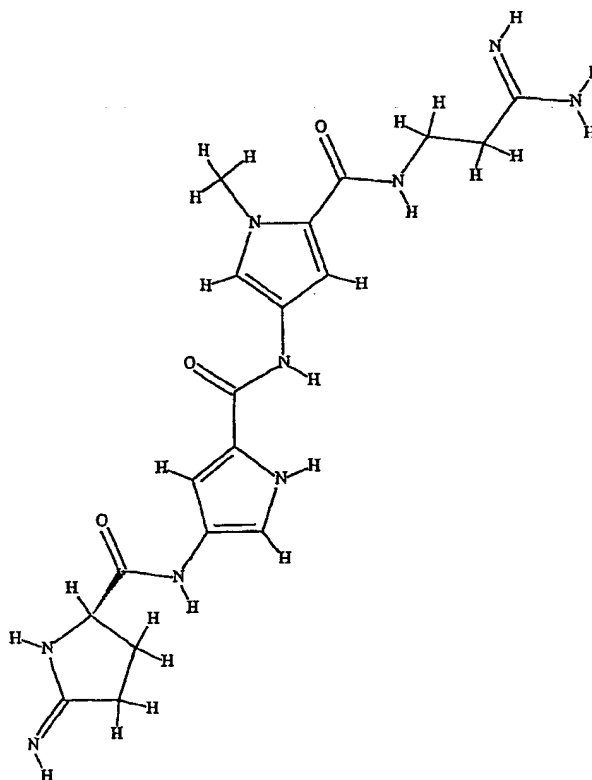
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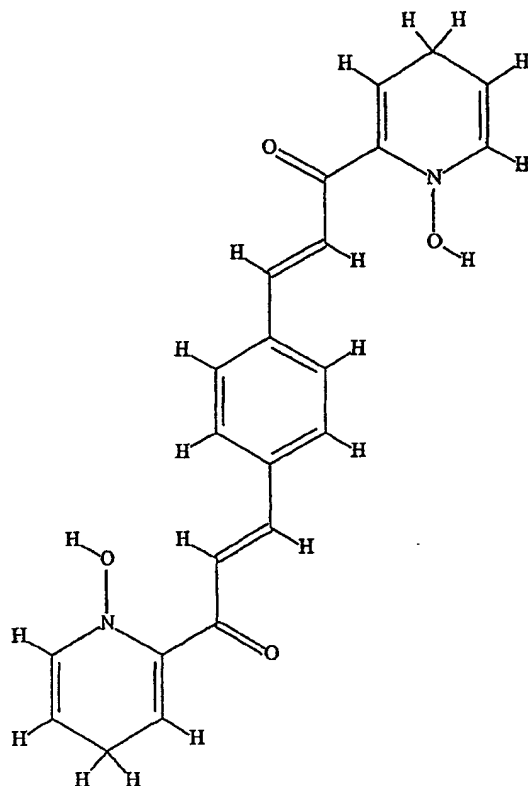


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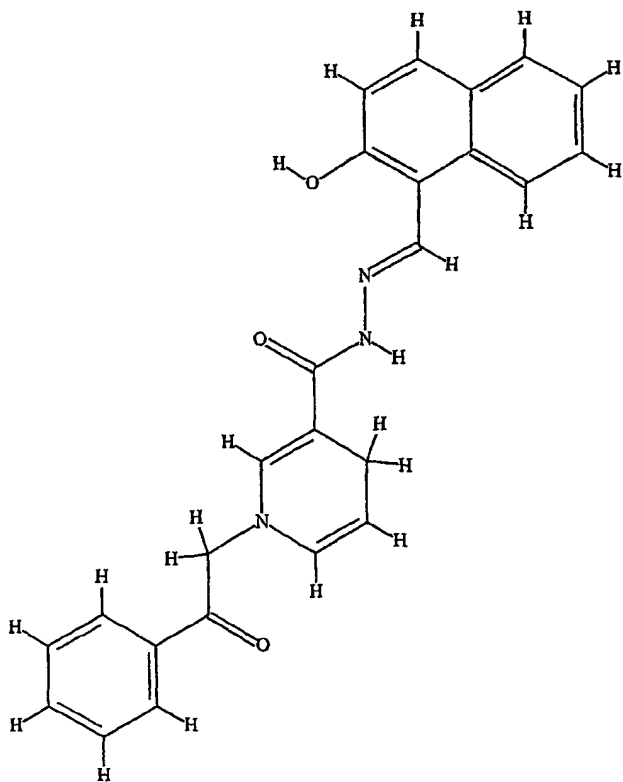


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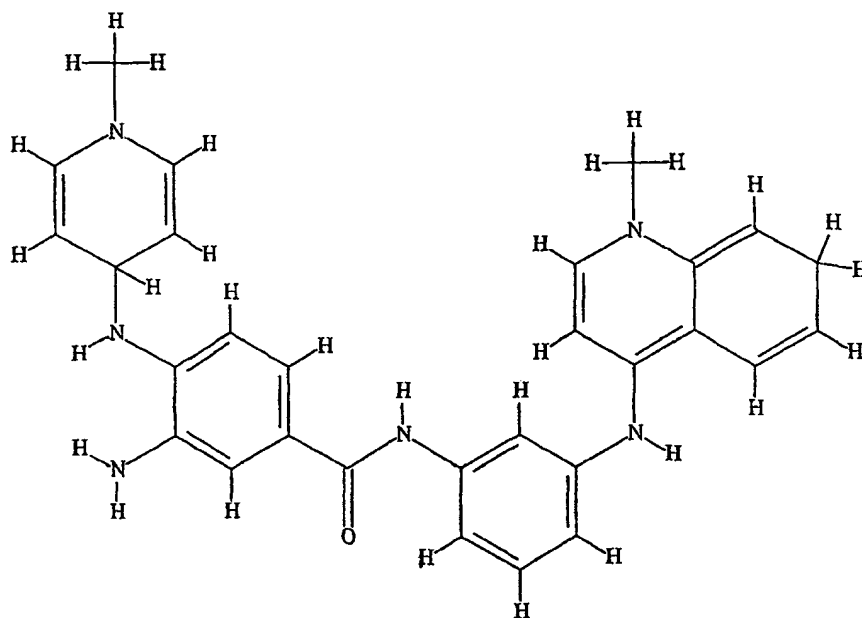


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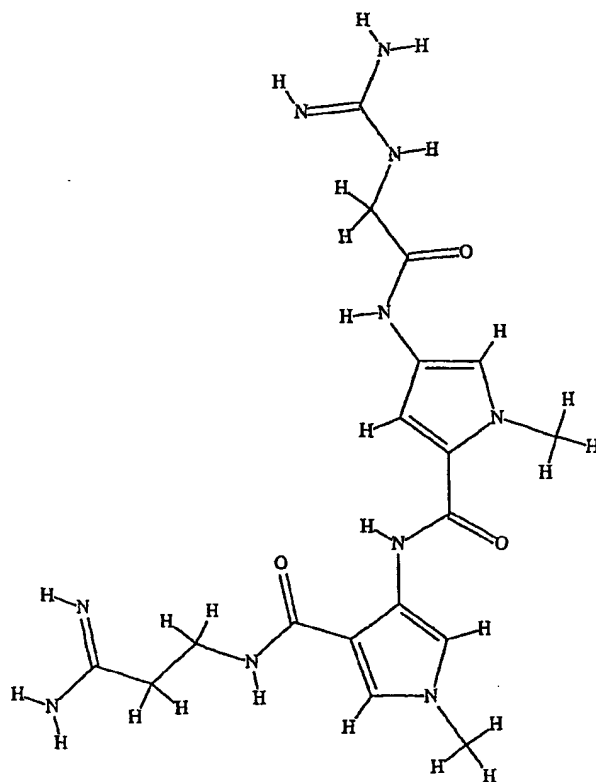
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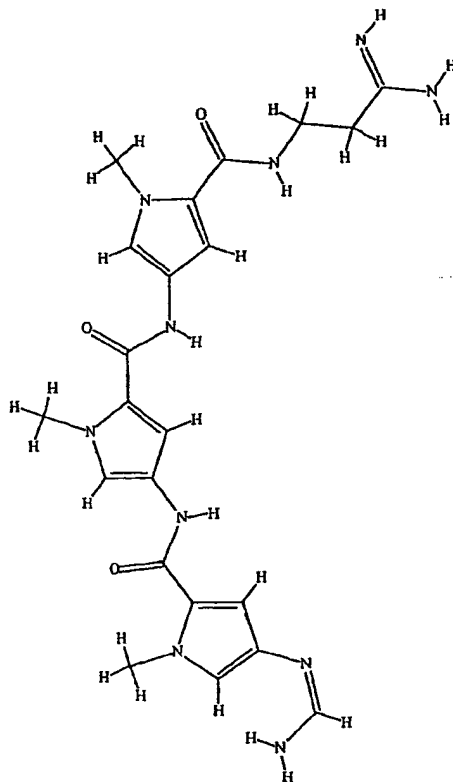


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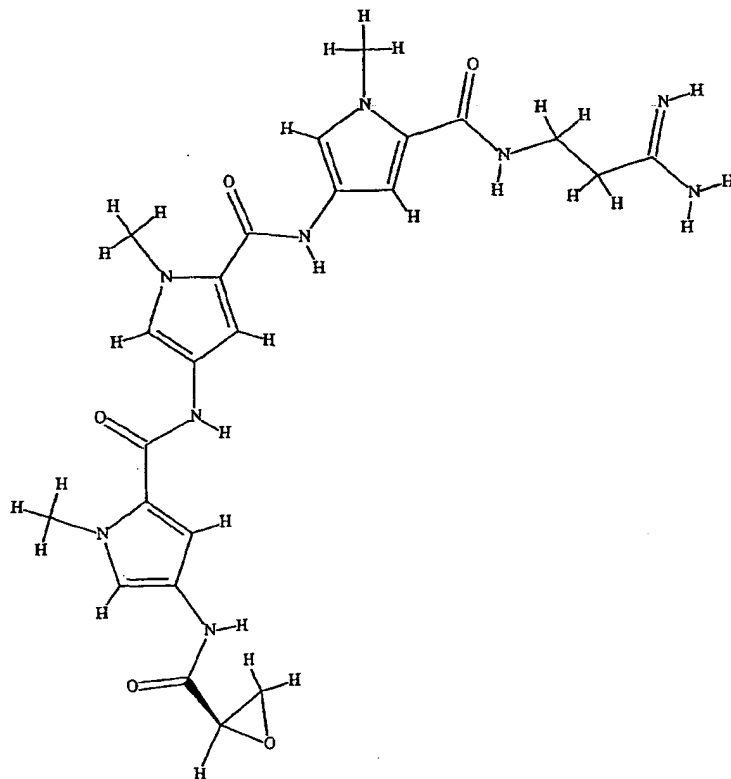


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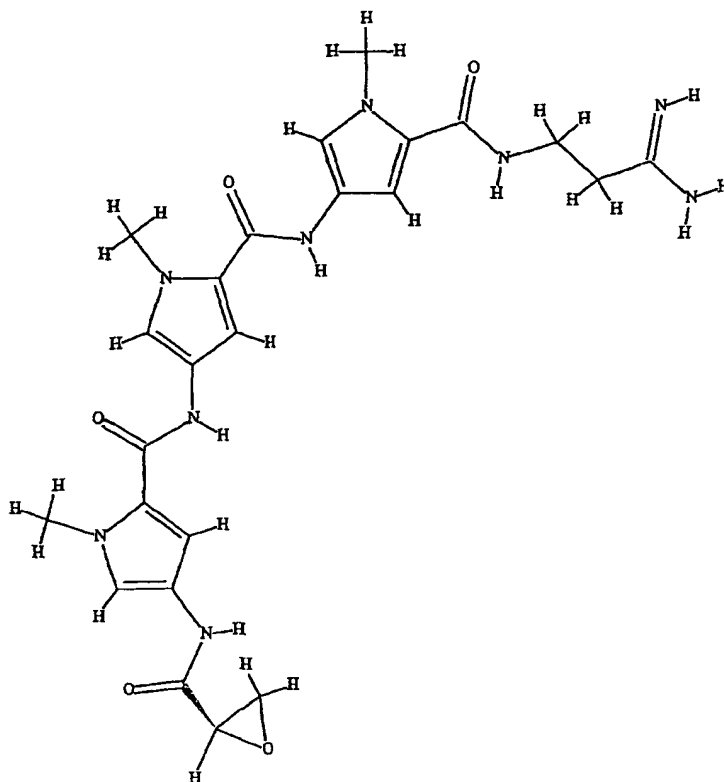


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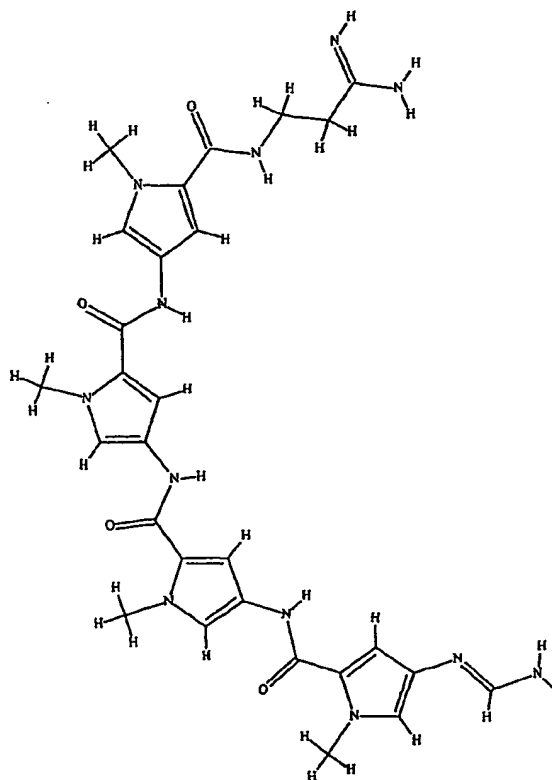


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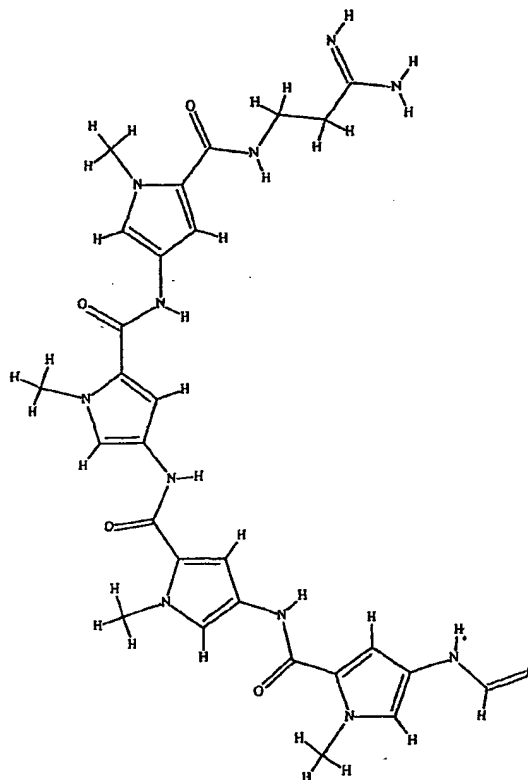


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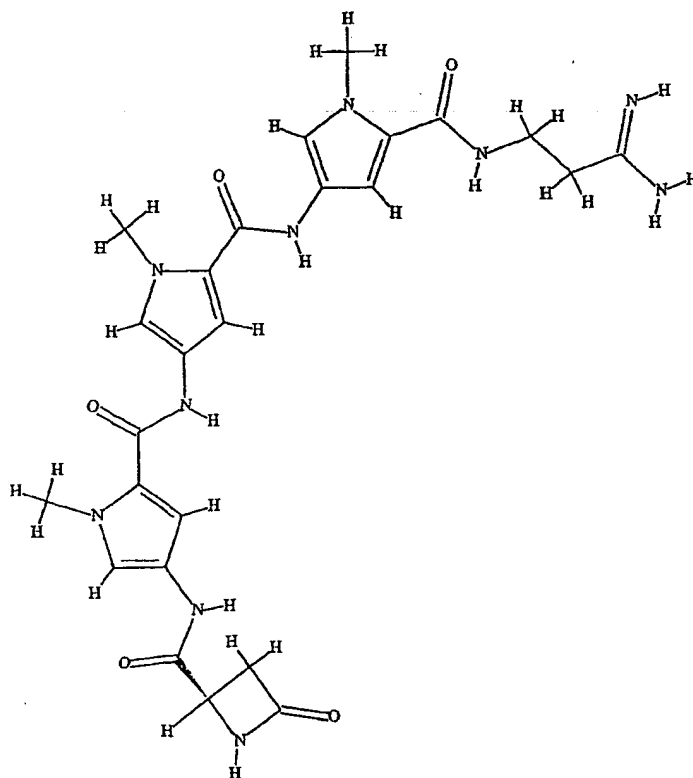


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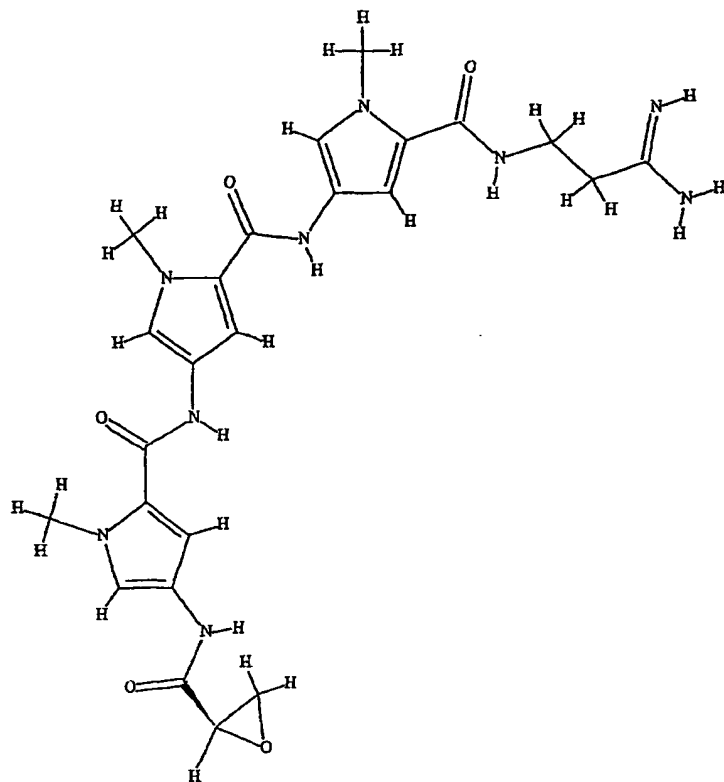


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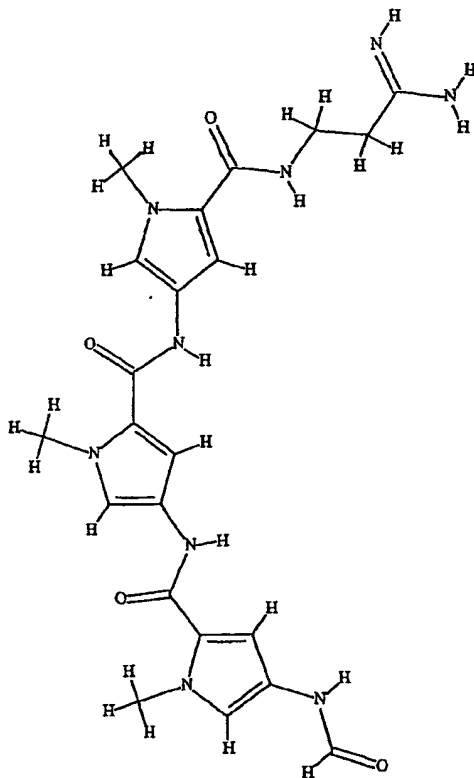


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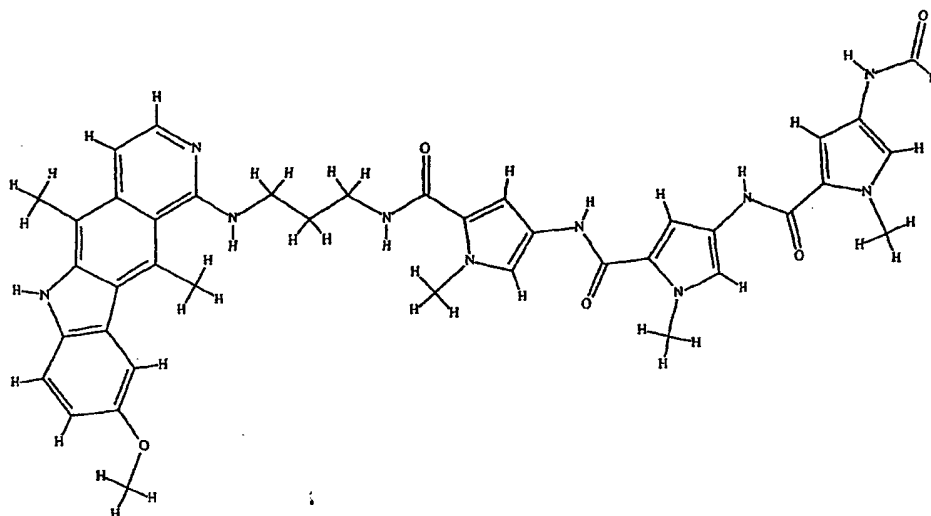


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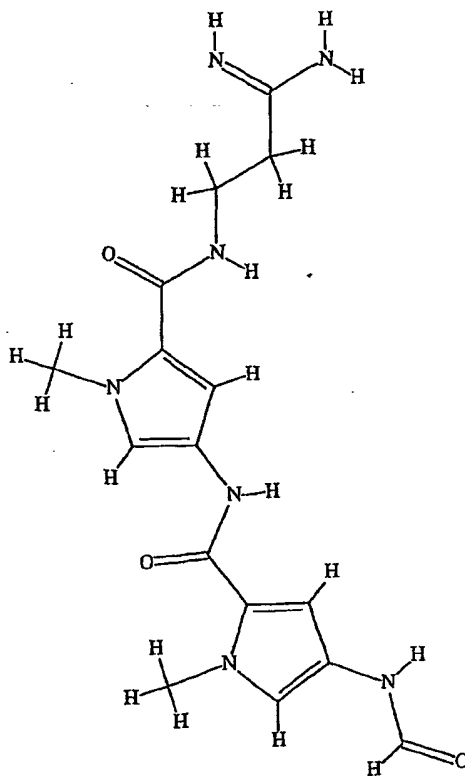




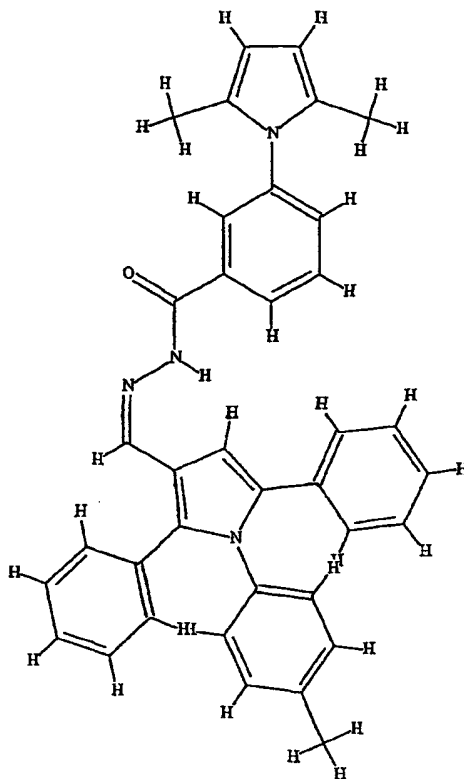
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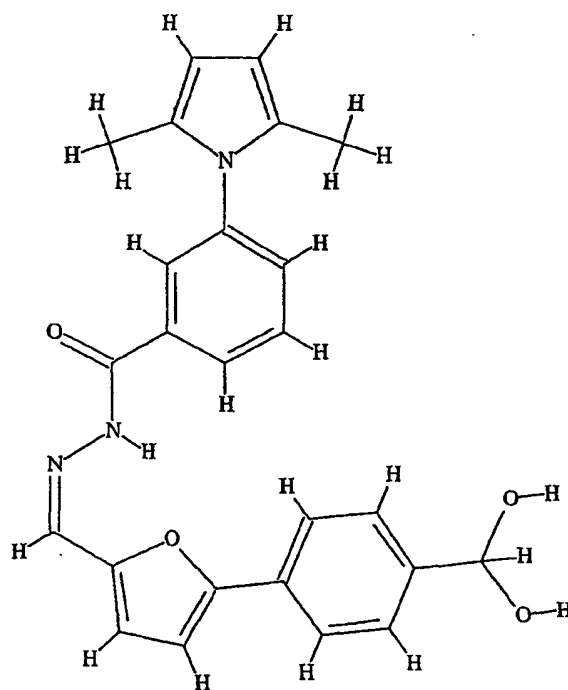
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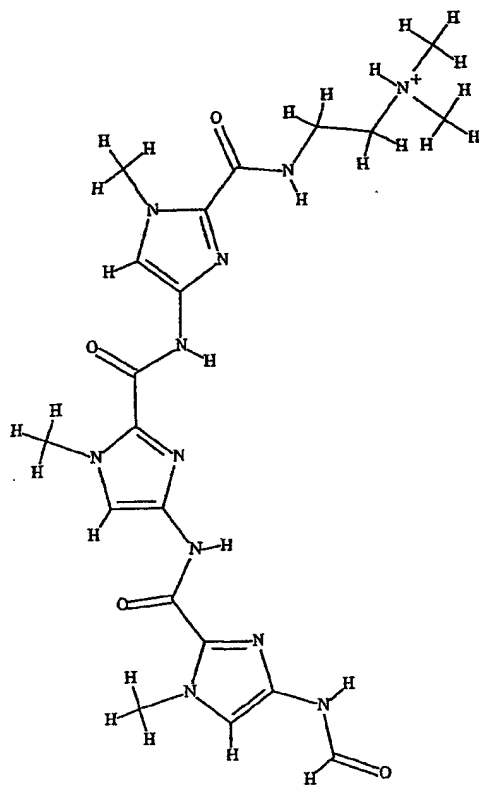
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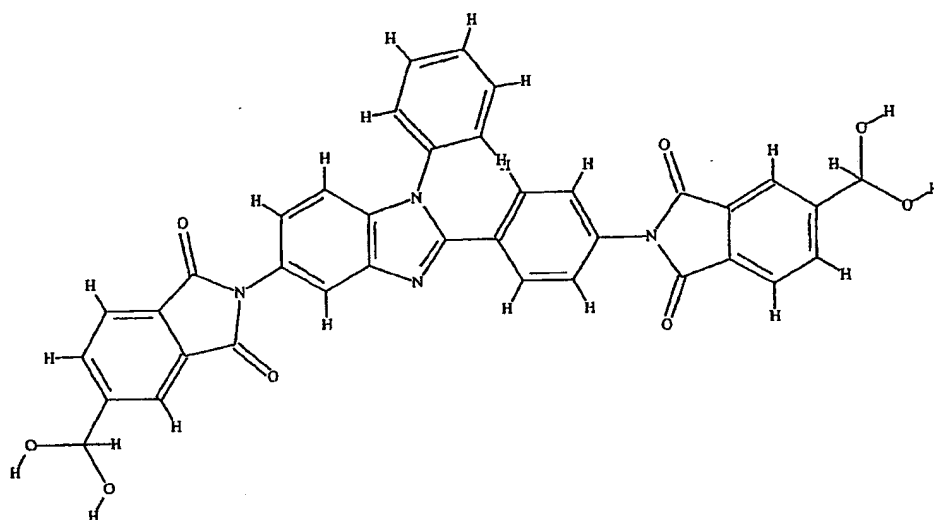
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248/248

495



496



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97074 Würzburg (DE). **BOGDAHN, Ulrich** [DE/DE];  
Talblick 27, 93055 Regensburg (DE).

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41, 50462 Köln (DE).

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(71) Applicant (*for all designated States except US*): **BIOG-  
NOSTIK GESELLSCHAFT FÜR BIOMOLEKU-  
LARE DIAGNOSTIK MBH** [DE/DE]; Ger-  
hard-Gerdes-Str. 19, 37079 Göttingen (DE).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SCHLINGEN-  
SIEPEN, Karl-Hermann** [DE/DE]; Pappelweg 3, 37120  
Bovenden-Lenglem (DE). **SCHLINGENSIEPEN,**  
**Reimar** [DE/DE]; Zur Scharfmühle 34, 37083 Göt-  
tingen (DE). **APFEL, Rainer** [DE/DE]; Eilsbrunner  
Str. 22, 93161 Sinzig (DE). **BRYSCH, Wolfgang**  
[DE/DE]; Brüder-Grimm-Allee 62, 37075 Göttingen  
(DE). **JACHIMCZAK, Piotr** [DE/DE]; Sterenstr. 37,

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(57) Abstract: A method for stimulating immune cells and/or the immune system, and/or reducing invasion and/or metastasis of  
tumor cells by inhibiting expression and/or functional activity of "Melanoma Inhibitory Activity" MIA.

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According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, MEDLINE, EMBASE, PAJ, SEQUENCE SEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	BOSSERHOFF A -K ET AL: "MELANOMA-INHIBITING ACTIVITY, A NOVEL SERUM MARKER FOR PROGRESSION OF MALIGNANT MELANOMA" CANCER RESEARCH,US,AMERICAN ASSOCIATION FOR CANCER RESEARCH, BALTIMORE, MD, vol. 57, no. 15, 1 August 1997 (1997-08-01), pages 3149-3153, XP002060476 ISSN: 0008-5472 the whole document --- -/--	1-23



Further documents are listed in the continuation of box C



Patent family members are listed in annex.

## \* Special categories of cited documents:

\*A\* document defining the general state of the art which is not considered to be of particular relevance

\*E\* earlier document but published on or after the international filing date

\*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

\*O\* document referring to an oral disclosure, use, exhibition or other means

\*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

28 November 2001

Date of mailing of the international search report

07/12/2001

Name and mailing address of the ISA

European Patent Office, P B 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel: (+31-70) 340-2040, Tx: 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Moreau, J

## INTERNATIONAL SEARCH REPORT

International Application No

PLT/EP 01/02695

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication where appropriate of the relevant passages	Relevant to claim No
X	JACHIMCZAK PIOTR ET AL: "Immunosuppressive effects of melanoma-inhibiting activity (MIA) - a possible role in tumor-host interactions." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, no. 41, March 2000 (2000-03), page 115 XP000971517 91st Annual Meeting of the American Association for Cancer Research.; San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X the whole document ---	1-23
A	EP 0 945 507 A (ROCHE DIAGNOSTICS) 29 September 1999 (1999-09-29) the whole document ---	1-23
A	JACHIMCZAK P ET AL: "Modulation of peripheral blood mononuclear cell (PMBC) activation by adhesion-regulating protein MIA." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 40, March 1999 (1999-03), page 79 XP000971516 90th Annual Meeting of the American Association for Cancer Research; Philadelphia, Pennsylvania, USA; April 10-14, 1999, March, 1999 ISSN: 0197-016X the whole document -----	1-23

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.2

Present claims 1-3, 5-8, 10-19 and 21-23 relate to a compound defined by reference to a desirable characteristic or property, namely the inhibition of the expression of the activity of MIA

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the product/compound/method/apparatus by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the compounds defined as antisense in claim 4 or as peptides described in claim 9.

Present claims 2,3, 5-8, 10-19 and 21-23 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds/products/apparatus/methods claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds defined as antisense in claim 4.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PLI/EP 01/02695

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
EP 945507	A	29-09-1999	EP 0945507 A1	29-09-1999
			AU 3599199 A	18-10-1999
			WO 9950411 A2	07-10-1999
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Form PCT/ISA/210 (patent family annex) (July 1992)